HUMAN LYMPHOID CELL IMMUNOPHENOTYPES

In situ immunochemistry of human lymphoid tissue, benign and malignant cutaneous lymphohistiocytic infiltrates with monoclonal antibodies.

by

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ABSTRACT

This thesis describes the results obtained with 36 monoclonal antibodies (McAbs) and immunocytochemistry on a) non-malignant lymphoid tissue [thymus (1)*, lymph node (5), tonsil (15)]. b) 104 skin biopsies of cutaneous lymphoma (40), contact dermatitis (14), parapsoriasis (17), chronic inflammatory dermatosis (9), non-lymphomatous erythroderma (3), atypical lymphocytic infiltrate (3), granulocytic sarcoma (1), lymphocytoma (3), lymphomatoid papulosis (3), lymphocytic infiltrate of Jessner (1) chronic lymphocytic leukaemia (1) , myelomonocytic leukaemia (1), histiocytosis X (3), and c) normal scalp (5).

In lymphoid tissue, determinants against T, and B cells (and their subsets), macrophages /D cells, and K/NK cells have a distinct topography. Immature thymic determinants OKT6, OKT9, OKT10 are identified in extrathymic locations (tonsil, and/or inflammatory infiltrates as well as lymphomas). OKT6 cross reacts with epithelial dendritic (Langerhans') cells. J5 (common acute lymphoblastic leukemia antigen) is absent in lymphoid tissue but present in some cases of mycosis fungoides. Immature myeloid markers (My10, My12) are absent in lymphoid tissue. The so called leukaemia/lymphoma markers Be1/Be2 react with follicular epithelium (Be1) , dermal endothelium (Be2), tonsil (Be2), benign dermatoses (Be1 and Be2) as well as most lymphomas. A mature helper T cell phenotype is usually present in cutaneous T cell lymphoma (CTCL) and inflammatory dermatoses. However, OKT9 and OKT10 are preferentially expressed in CTCL versus parapsoriasis and benign infiltrates.

Lymphomas of non-mycosis/Sezary type can be categorized into T, B and U types. They often show aberrant differentiation when examined with multiple markers.

Pseudolymphomas, sarcoidosis, leukaemia and histiocytosis X show characteristic phenotypes denoting the expansion of selected lymphohistiocytic subpopulations. The histiocyte of histiocytosis X predictably expresses the OKT6 marker, but in addition reacts with the Leu3A (helper T cell) antibody.

Characteristic dendritic cell markers are associated with B (R423+) and T (OKT6+) populations in lymphoid tissue and skin.

K/NK cells are identified in benign and malignant infiltrates. This may have immuno-pharmacological application.

The markers used amply illustrate the heterogeneous nature of benign and malignant lymphoid populations.

*(n) = number of specimens tested.

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ABBREVIATION

ACD	-allergic contact dermatitis
AD	-atopic dermatitis
ALL	-acute lymphoblastic leukaemia
AML	-acute myeloid leukaemia
ANL	-acute nonlymphocytic leukaemia
APC	-antigen presenting cell
B cell	-bursal or bone marrow derived cell
BFUE	-burst forming units - erythroid
C3	-third component of complement
CALLA	-common acute lymphoblastic leukaemia antigen
CBCL	-cutaneous B cell lymphoma
CBD	-chronic benign dermatoses
CE	-crypt epithelium
CFUC	-colony forming units - culture
CFUE	-colony forming units - erythroid
CLL	-chronic lymphocytic leukaemia
CTCL	-cutaneous T cell lymphoma
D cell	-dendritic cell
DAB	-diaminobenzidine
DHS	-delayed hypersensitivity
DNA	-deoxyribonucleic acid
DRC	-dendritic reticulum cell
E Rosette	-erythrocyte rosette
EAC	-erythrocyte antibody complement
EM	-electron microscopy
FDC	-follicular dendritic cell
GC	-germinal centre
H/S	-helper/suppressor ratio
H202	-hydrogen peroxide
HCL	-hydrochloric acid
HLA	-human leukocyte antigen
HTLA	-human T lymphocyte antigen
HTLV	-human T cell lymphoma virus
HX	-histiocytosis X
IFA	-interfollicular area
IgG	-immunoglobulin G
IgM	-immunoglobulin M
K cell	-killer cell
K/L	-Kappa/Lambda ratio
LAC	-large atypical cell
LCL	-large cell lymphoma
LF	-lymphoid follicle
LIJ	-lymphocytic infiltrate of Jessner
LN	-lymph node
LP	-lichen planus
LPAP	-large plaque atrophic parapsoriasis
Ly Pap	-lymphomatoid papulosis
McAb	-monoclonal antibody
MF	-mycosis fungoides
MHC	-major histocompatibility complex

MML	-myelomonocytic leukaemia
MPS	-mononuclear phagocyte system
NHL	-non-Hodgkin's lymphoma
MZ	-mantle zone
NK cell	-natural killer cell
NSE	-non-specific esterase
PAP	-peroxidase anti-peroxidase
PBL(S)	-peripheral blood lymphocyte(s)
PBS	-phosphate buffered saline
PHA	-phytohaemogluttinin
PPD	-purified protein derivative
RS	-Reed-Sternberg
SALT	-skin associated lymphoid tissue
SIg	-surface immunoglobulin
SS	-Sezary syndrome
T cell	-thymic derived cell
TCR	-T cell receptor

CHAPTER ONE

FOREWORD

In 1968 I heard of the tragic death of a school classmate who was struck down by acute leukaemia. He was a gifted pupil and his death caused a shudder in the existence of many of us who were enjoying a relatively carefree sixth year in school. Around the same time I learned that my father, a doctor, had developed an enlarged spleen from a "reticulosis." He courageously continued to look after his patients right up until his death four years later. The loss of these two people at such an inappropriate time in their lives repeatedly stimulated questions as to the nature of lymphoproliferative disorders, questions so complex that they barely seemed to be touched in standard texts.

In 1972 Professor (later Sir Alastair) Currie offered me the opportunity of spending an additional year in Pathology to study diseases more thoroughly than was possible in the basic curriculum. A stimulating year was spent with Doctor (later Professor) W. L. Ford whose attention was focused on the Lymphocyte; during that time (after some initial resistance) I learned that knowledge of the normal is very important when studying the abnormal cell. Work was being conducted on the in vivo migration patterns of normal and malignant lymphocytes. While reading basic science literature on cell differentiation and host tumour interactions I became interested in the notion that the mass of lymphoid cells in lymphomatous lymph nodes and spleen might be more than a homogeneous proliferation of lymphoma cells and might contain a variety of cancer cells at various stages of development mixed with host reactive cells. However, at that time markers to examine the different Phenotypes of inflammatory cells were sparse. A

chromosome marker of the transplantable lymphoma I was studying was discovered (McMillan 1973) but this was of limited use in providing the type of information I required. A more ideal method would have been to study cells in tissue section with markers tagging tumour and reactive lymphocytes in a manner analogous to radioactive labelling studies of lymphocyte migration. However, the number of markers at that time was limited, even more so in human disease where Doctor (later Professor) Angus Stuart and Doctor John Habeshaw were beginning to apply the newly discovered E rosettes to peripheral blood cells and lymphoid tissue to identify T lymphocytes in a variety of pathological states. Because of limited technology and the desire to continue medical school and post graduate studies this idea lay dormant for 8 years. During that time further questions arose on seeing patients in the wards with leukaemia-lymphoma and rare disorders such as histiocytosis x. In 1978 I entered Dermatology hoping to combine patient care with a side interest in pathology. In 1980 I headed for the University of Oklahoma to complete their training programs in dermatology and dermatopathology. However, the chairman of the department, Doctor Mark Everett, informed me that I should also work with T cells, preferably in mycosis fungoides and parapsoriasis, since these conditions were one of the clinical interests of the department and, as already indicated, interested me. I was also informed that an empty laboratory was waiting in the pathology department. Since no one on campus had closely related interests, he advised me to learn as much as possible prior to studying in Oklahoma. Basic immunocytochemical techniques were learned at the University of Glasgow, and University of London. Alternative methods of cell analysis (DNA cytophotometry) were also reviewed at the University of Lieden. While in London I learned that new reagents for T cell identification (Monoclonal Antibodies) would be available soon and several months

later, while doing the experiments with an anti-T cell serum, noticed an advertisement in an immunology journal for such markers. Despite their expense, my chairman kindly agreed to let me purchase some.

After an introduction to the lymphocyte, its identification and the purpose of the work, this thesis will describe and discuss the results obtained.

CHAPTER TWO

INTRODUCTION - THE LYMPHOCYTE AND ITS IDENTIFICATION

Including:

A. Early Concepts of Lymphoma

B. Discovery of T and B Lymphocytes in Animals and Humans

C. "Null" Cells and Other Subpopulations

D. The Mononuclear Phagocyte System and D Cells

E. Cell Differentiation and Lymphoma

F. Early Application of Surface Markers and Cytochemistry to Human Tissue

G. Monoclonal Antibodies

H. Objectives of Work Described in Thesis

CHAPTER TWO

INTRODUCTION - THE LYMPHOCYTE AND ITS IDENTIFICATION

A. EARLY CONCEPTS OF LYMPHOMA

The background to this work is dependent on a fusion of modern pathological concepts of lymphoma and lymphocyte immunology. Hodgkin's paper of gross pathology of the lymphoid organs was published in 1832 (Hodgkin, 1832). The use of the microscope in the 1830's led to Schwann's theory that all animals were composed of elementary units called cells (Schwann, 1839) and to Virchow's idea of "cellular pathology" (Virchow, 1863). The combination of gross and microscopic observations led to the splitting of diseases of white cells into those with high circulating white blood cell counts (Craigie 1845; Bennet 1845) and those with generalized lymphadenopathy and splenomegaly but without increased levels of circulating white blood cells (Wunderlich, 1858). Early on, Virchow recognized the difficulty in separating lymph node malignancy from hyperplasia (Virchow, 1864-1865). Initial classifications were clinical but, later, histologic observation led to the subdivisions of pseudoleukaemia (aleukaemic-leukaemia), lymphosarcomatosis (lymphoma) and lymphadenoma (Hodgkin's disease) (Wunderlich, et al., 1858). Roulet's observation (Roulet, 1930) of reticulum cell sarcoma of lymph nodes resulted in the division of lymphoid malignancies into the more familiar categories of lymphosarcoma, Hodgkin's disease and reticulum cell sarcoma (Ghon and Roman, 1916). In 1942 Gall and Mallory further emphasized the importance of histology by dividing lymphosarcoma and reticulum cell sarcoma into more and less differentiated categories and added follicular lymphoma to their scheme (Gall and Mallory, 1942). Subsequently, the systems of Rappaport (Rappaport, 1966a) the British National Lymphoma Investigation (Bennett, et al., 1974) and Dorfman (Dorfman, 1974) appeared.

Immunologic advances paved the way for further developments.

B. DISCOVERY OF T AND B LYMPHOCYTES IN ANIMALS AND HUMANS

In 1956 Glick described the role of the bursa of Fabricius in the development of humoral immunity in the chicken (Glick, et al., 1956) and subsequently the mammalian thymus was shown to have a pivotal role in immunological competence (Fichtelius, et al., 1961; Miller, 1961; Good, et al., 1962).

Further work was conducted in the chicken because of the simultaneous presence of thymus and bursa in this animal, and a functional dissociation of the chicken immune system based on differences in thymic and bursal influences was suggested (Szenberg, 1962).

Ablative studies in laboratory animals (Warner, 1965; Cooper, et al., 1966) and clinical studies in patients with congenital thymic dysplasia or aplasia (Nezelof, et al., 1964; Digeorge, 1968) demonstrated that cells derived from the thymus, T cells, are the mediators of cellular immunity including delayed hypersensitivity, allograft rejection, and graft versus-host disease. Parallel studies in chickens, rodents and hypogammaglobulinaemic children revealed that cells responsible for antibody production, B cells, undergo thymus independent generation in the avian bursa of Fabricius and in mammalian foetal liver and bone marrow. (Gitlin, et al., 1959; Warner, 1965; Cooper, et al., 1966a)

The importance of thymic and bursal influences in pathology became clear when thymectomy and bursectomy were found to have a suppressive effect on the development of lymphomas in mice and chickens. (Gross, 1960; Moloney, 1960).

In 1966 Cooper and colleagues proposed that lymphoid malignancies might be usefully classified as either thymus system or immunoglobulin - producing system malignancies (Cooper, 1966b). The clinical application of this theory would however require the development of surface markers

characteristic of the B and T systems. The demonstration of surface immunoglobulin on rabbit lymphocytes (Sell and Gell, 1965) was followed by similar studies (Raff, et al., 1971; Pernis, et al., 1971) in mice, chickens and rabbits. Their bursal derivation was shown in chickens; (Rabellino and Grey, 1972) whereas, the thymus was shown to be the source of surface immunoglobulin negative cells (Unanue, et al., 1971). Similar approaches were used in human peripheral blood lymphocytes in normal and pathological states (Grey, et al., 1971) Although human T cells lacked the theta antigen which had been useful in mouse systems, (Raff, 1969) the demonstration of rosetting capacity with sheep red cells from 1970 onwards (Brain, et al., 1970; Coombs, et al., 1970) permitted their quantification in a variety of conditions (Wybran and Fudenberg, 1973).

C. "NULL" CELLS AND OTHER SUBPOPULATIONS

Immunologic characterization of lymphomas was then correlated with their histology (Braylan, et al., 1977; Lukes, et al., 1978a & b; Stein, 1978). However, physiological studies had also revealed a third major division of lymphoid cells the non-T, non-B or "Null" lymphocytes (Greenberg, et al., 1973). This population is composed of stem cells, killer (K) cells which kill target cells only when the latter are coated with antibody, and natural killer (NK) cells which are cytotoxic in the absence of antibody (Vogler, et al., 1979). This suggests the presence of an additional component of potential "reactive" cells which might be studied in lymphoproliferative disorders.

The demonstration of T cell subpopulations with "helper" and "suppressor" activity (Moretta, et al., 1977) also adds a further dimension of sophistication in the analysis of Lymphoid populations.

D. THE MONONUCLEAR PHAGOCYTE SYSTEM AND D CELLS

The analysis of lymphoid subpopulations would, of

course, not be complete without consideration of the mononuclear phagocyte system (MPS).

The MPS is widely distributed throughout the body and is derived from the bone marrow monocyte (Van Furth, et al., 1972). The cells of this system include monoblasts, promonocytes, monocytes, macrophages (histiocytes), multinucleated giant cells, osteoclasts, and microglial cells of the central nervous system.

Metchnikoff (Metchnikoff, 1892) pointed out that an essential factor of inflammation was phagocytic activity of microphages and macrophages. Kiyono (Kiyono, 1914) suggested the term "histiocyte" to describe phagocytic cells in lymph nodes, spleen and connective tissue. Aschoff (Aschoff, 1924) proposed the term reticuloendothelial system (RES) to cover endothelial cells and reticulum cells which were also thought to be related to histiocytes. Putative proliferations of this system have been labelled "reticulosis" (Robb-Smith, 1938) and "histiocytosis" (Rappaport, 1966b).

Although rare, the possible presence of true histiocytic proliferations and the role of the MPS in immune responses and cell mediated cytotoxicity suggests that the study of an MPS component in lymphoid infiltrates would be worthwhile. Dendritic cells (Tew et al., 1982) (D cells), although showing little or no phagocytic activity, are probably a subset of the MPS and require to be considered for similar reasons.

E. DIFFERENTIATION AND LYMPHOMA

The concept of "differentiation" may also be important in characterizing lymphoid subpopulations including lymphomas. Failure of differentiation could result in the accumulation of an immature counterpart of the normal cell (differentiation block) without a significant change in proliferation rate (McGrath, 1981). A stem cell disorder with increased fraction of proliferating cells but

relatively normal subsequent differentiation (expansion of a single clone) would result in increase in size of all subsequent differentiation compartments. The amplifications of the differentiation pathway would result in the more differentiated compartments being larger (McGrath, 1981). T cells normally undergo intrathymic maturation (Mathieson and Fowlkes, 1984) and subsequently seed to peripheral organs. A neoplasm such as mycosis fungoides which appears to begin peripherally (although a subject of some controversy) (Ryan, et al., 1973) would therefore be particularly worthy of study if differentiation markers were available. An additional possible facet of malignancy (not mentioned in McGrath's hypothesis) is the presence of phenotypes not found during normal ontogeny (aberrant differentiation).

If technically feasible, then a framework incorporating typing of lymphoma populations for T, B, macrophage, D cell, K/NK content could be envisaged. Parallel studies of non-malignant lymphoid populations (eg. lymphoid organs unaffected by lymphoma and non-malignant lymphocytic infiltrates in skin) would be useful for comparison. Any additional information on differentiation status would be a further bonus.

The progress in this area up until 1980 will now be described, with special emphasis given to cutaneous pathology.

F. EARLY APPLICATION OF SURFACE MARKERS AND CYTOCHEMISTRY TO HUMAN TISSUE

In the early 1970's T cells, B cells and monocytes were identified by their respective receptors for sheep erythrocytes (T cells), C3 [B cells (Bianco, et al., 1970) and monocytes (Huber, et al., 1968)] and Ig G (monocytes) (Abramson, et al., 1970). Extracted cells were studied in cell suspensions of lymphoid tissue (Claudy, et al., 1976)

and skin affected by benign and malignant lymphocytic infiltrates (Tan, et al., 1975).

In situ studies utilizing similar technology were performed in comparable conditions (Dukor, et al., 1970; Silveira, 1972; Edelson, et al., 1973). In situ Techniques did not require such dense populations as extraction methods, theoretically at least did not damage cells to the same extent, and provided some architectural information. Some information on lymphoid maturity could be obtained by E rosetting with and without neuraminidase (Stingl, et al., 1977) and lymphomas have been categorized into "early" and "late" thymocyte types by this method (Yamanaka, et al., 1981). The in situ E rosetting method was difficult to perform (Brubaker and Whiteside, 1977) and could not be adapted for ultrastructural use.

Cellular identification expanded with the development of anti-T (Yata, et al., 1970) and anti-B (Brochier, et al., 1976) cell sera. Cell subpopulations were then identified in blood, (Brouet, et al., 1973) lymphoid tissue, (Schmitt, et al., 1976) and skin (Schmitt, et al., 1976a,b; Claudy, et al., 1977; MacDonald, et al., 1978). These studies utilized in situ or suspension methods (immunofluorescence and immunoperoxidase) both at the light and electron microscopic level. Each of the various approaches used had its own advantages. The immunoperoxidase technique did not require a specialized microscope, provided more permanent

preparations and better visualization of tissue architecture (Petts and Roitt, 1971) as well as being applicable at the ultrastructural level (Schmitt, et al., 1976a,b). However each investigator can usually list advantages of his own favourite technique.

Enzyme cytochemical techniques have also been successfully used to demonstrate cell types in cutaneous lymphomas of T (Flandrin and Daniel, 1974; Sterry, et al., 1980) and B type (Burg and Braun-Falco, 1978). Enzyme activity, however, tends to be variable in malignant T cells (Chu, et al., 1981).

G. MONOCLONAL ANTIBODIES

One problem associated with the use of heteroantisera is their inherent lack of specificity (Heyderman, 1979) despite multiple absorptions; although, one unique marker has been claimed to reside on Sezary cells as detected by antisera (Khan, et al., 1980). An ideal method of tissue identification of lymphoreticular cells adapted from a recent review (McMillan, 1985) is listed in Table 1. Since the introduction of the hybridoma technique in 1975 by Kohler and Milstein (Kohler and Milstein, 1975) high-titre specific monoclonal antibodies (McAbs) have been produced against T cells (Engleman, et al., 1975), T cell subsets (Kung, et al., 1979a; Ledbetter, et al., 1981), immature T cells (Kung, et al.,1980), B cells (Nadler, et al., 1981a), monocytes (Todd, et al., 1981) and granular lymphocytes with

TABLE 1

Tissue identification of lymphoreticular subpopulations -
Requirements of the Ideal Method (Adapted from McMillan, 1985)

1. Simple
2. Rapid
3. Inexpensive:
    a. reagents
    b. microscope
4. Demonstration of lymphoreticular cells of varying maturity and subsets of mature cells
5. Permanent record provided
6. Specific
7. Applicable to ultrastructural studies
8. Provides information on in vivo relationships
9. Produces minimal damage to cells and antigens
10. Reagents widely available
11. Easily quantifiable
12. Simultaneous demonstration of multiple antigens
13. Applicable to Paraffin Sections

NOTE: Prior to 1980 existing in situ immunoperoxidase technology satisfied several conditions above (Nos. 1,2,3,5,7,8,9). The advent of Monoclonal antibodies permitted improvements in Nos. 4, 6 and 10; although, initially to some detriment in 3a as the original commercially available reagents were generally more expensive than heteroantisera. However, this was offset, to some extent, by the ability to use higher dilutions with these reagents.

killer-natural killer function (Abo and Balch, 1981). Many of these reagents became commercially available in 1980-1983 and set the stage for a new chapter in the identification of lymphoid cells. If found to be applicable to tissue sections these reagents, with their increased range of detection, could be added to the existing immunoperoxidase technology.

H. OBJECTIVES

The objectives of the work described in this thesis are as follows:

1) To determine

a) whether Monoclonal determinants expressed on T cells (and their subsets), B cells, K/NK cells, monocytes/macrophages, and D cells are readily demonstrable in non-malignant lymphoid tissue (i.e. tonsil, lymph node, thymus) and skin.

b) whether these determinants have a recognizable topography.

c) whether so called "immature determinants" are truly restricted to thymus and bone marrow as indicated in original studies of their production or if they might be found in peripheral lymphoid organs, or normal skin.

2. To study the immune phenotype of allergic contact dermatitis. This would serve as a prototype of reactive inflammatory states.

3. To study the Phenotype of cutaneous T cell lymphoma

(CTCL), i.e., Mycosis Fungoides and Sezary Syndrome, with respect to:

a) the presence of markers associated with mature T cells and their subsets. CTCL has previously been shown to be a neoplasm of helper T cells by functional studies. (Berger et al., 1979) The latter are, however, difficult to perform, thus limiting their use. This raises the question whether Monoclonal subset studies might demonstrate CTCL to consist of a phenotypically monomorphous expansion of helper T cells. This would have important implications in diagnostic pathology where the discrimination of benign from malignant lymphocytic infiltrates is often difficult.

b) the presence of immature determinants. According to the differentiation models of neoplasia already mentioned (page 26) one might expect to find cells in CTCL expressing immature antigens, aberrant differentiation, or proliferation associated markers. Again this could be diagnostically important.

c) simultaneous comparison of CTCL and prelymphomatous disorders, eg., large plaque parapsoriasis (poikiloderma vasculare et atrophicans). The latter group exhibits clinical heterogeneity with approximately 10% of cases converting to lymphoma (Everett, 1978). Similar phenotypic heterogeneity might serve to identify cases with the propensity for lymphoma development. Comparison with reactive states such as allergic contact dermatitis, and

lichen planus would be an integral part of such studies.

4) To determine whether cutaneous lymphomas of non-Hodgkin's type might

a) be categorized by Monoclonal antibodies into T, B or true histiocytic (monocyte - macrophage) subtypes.

b) show evidence of disturbed differentiation through their pattern of expression of markers normally found on mature and immature cells.

5) To examine whether characteristic phenotypic patterns or expansions occur in other cutaneous disorders, eg., those reputedly involving cells of the mononuclear - phagocyte system (MPS) (Histiocytosis X, sarcoidosis), and in particular whether these disorders involve expansions of any recognizable MPS subset. Examination of leukaemias involving lymphocytes/myeloid cells (chronic lymphocytic leukaemia/myelo-monocytic leukaemia) and pseudo-lymphomas (lymphocytoma cutis, lymphocytic infiltrate of Jessner, Lymphomatoid papulosis) would provide further phenotypic comparisons between malignant and benign disorders.

6) To scrutinize lymphomatous infiltrates for the presence of putative host reactive cells such as macrophages or K/NK cells. This might be of prognostic or therapeutic importance.

7) To compare the reactivity of non-malignant lymphoid tissue, benign and malignant cutaneous lymphocytic infiltrates with reagents stated to be relatively specific

for lymphoma cells.

This range of work was possible (and necessary) because of the novel nature of the reagents and lack of studies involving their use on tissue sections.

CHAPTER THREE

MATERIALS AND METHODS

Including:

A. Introduction
B. Tissues
C. Immunoperoxidase Method
D. Antibodies
E. Controls
F. Quantification
G. Alpha-Naphthyl Acetate Esterase Stain.

CHAPTER THREE

MATERIALS AND METHODS

A. INTRODUCTION

The in situ staining technique used was a modification of the indirect immunoperoxidase method (Heyderman, 1979) previously used successfully with heteroantisera for the identification of T cells in tissues. (Claudy, et al., 1977) The first cutaneous study showing this was practicable with monoclonals used lichen planus as a model (McMillan, et al 1981a).

B. TISSUES

Punch biopsies (3mm or 4mm) were obtained from clinically affected skin after 1% Xylocaine Epinephrine (Adrenaline) anaesthesia. A minimum of 2 biopsies was taken from each area tested, one submitted for routine haematoxylin and eosin pathology and the other treated separately for immunoperoxidase studies.

Surgically removed human thymus, lymph node, and tonsil were obtained as soon as possible after excision from patients at the Oklahoma Health Science Center and Saint Anthony's Teaching Hospitals, Oklahoma City, Oklahoma.

The 104 skin biopsies included 5 "normal" scalp from hair transplant patients (free of lymphocytic infiltrate but from individuals with androgenic alopecia), 14 allergic contact dermatitis, 24 mycosis fungoides, 3 Sezary Syndrome, 17 large plaque atrophic parapsoriasis (poikiloderma vasculare et atrophicans), 4 lichen planus, 3 atopic dermatitis, 2 chronic dermatitis of unspecified type, 13 non-Hodgkin's lymphomas of non-mycosis fungoides non-Sezary type, 3 non-lymphomatous erythrodermas, 3 atypical lymphocytic infiltrates, 1 granulocytic sarcoma, 3 lymphocytoma cutis, 3 lymphomatoid papulosis, 1 lymphocytic

infiltrate of Jessner, 1 chronic lymphocytic leukaemia, 1 myelomonocytic leukaemia, 3 histiocytosis X. Non-malignant lymphoid tissue consisted of 1 thymus, 5 lymph nodes, and 15 tonsils.

The clinical and pathological diagnoses were made by Board Certified Dermatologists and Dermatopathologists at Oklahoma University and light microscopic haematoxylin and eosin slides were reviewed by the authour (also certified in Dermatopathology).

Upon receipt, specimens were mounted on cork blocks with OCT medium (AMES Company, Elkhart, Indiana). The mounted tissue was then snap frozen in liquid nitrogen with quenching in 2-methyl butane. Tissue was stored at minus 70 degrees centigrade in an ultra-cold freezer (Revco, West Chester, Pennsylvania) until used.

C. IMMUNOPEROXIDASE METHOD

Cryostat sections (5 microns) were then processed as follows:

1. Fixation

Because of variable results obtained using 3% paraformaldehyde (McMillan, et al., 1981a; McMillan, et al., 1981b; McMillan, et al., 1981c) (which was previously used with T cell heteroantisera) (Chu et al., 1979) it appeared that the antigens detected by McAbs were more sensitive to the deleterious effects of fixation than the markers detected by heteroantisera. A full range of fixatives was therefore tested on normal lymphoid tissue and skin. The results have been published in detail elsewhere (McMillan, et al., 1982a). These involved testing the following for various lengths of time and temperature: acetone, paraformaldehyde (3%) in phosphate buffered saline (PBS), glutaraldehyde (0.1%) in PBS, ethyl alcohol (70%) in water, no fixative, methyl alcohol-hydrogen peroxide-hydrochloric acid mixture. Acetone fixation (suggested by Dr. David Mason, John Radcliffe Infirmary, Oxford England) at 4

degrees centigrade for 15 minutes was found to be optimal.
2. Rinsing in phosphate buffered saline (PBS), pH 7.4 for 30 minutes.
3. Application of reconstituted primary antibody, at optimal dilution in PBS at room temperature for 30 minutes. The 36 monoclonal antibodies used, their specificities, and sources are given in Table 2.
4. Rinsing in PBS for 30 minutes.
5. Application of secondary antibody, peroxidase-conjugated goat antimouse IgG or IgM (Tago, Inc., Burlingame, California) depending on the nature of the primary antibody; this was used at optimal dilution in PBS at room temperature for 30 minutes. The anti-IgG preparation is specifically formulated for use with monoclonal reagents and consists of a mixture of affinity-isolated antibodies directed against the four classes of mouse IgG (Johnson RC, Tago Inc. personal communication).
6. Rinsing in PBS for 20 minutes.
7. Application of Tris HC1 buffer, pH 7.6 for 10 minutes.
8. Application of diaminobenzidine (DAB) (Graham and Karnowsky, 1966) 10 mg per 10 ml of Tris HC1 buffer with 0.01% hydrogen peroxide for 10 minutes.
9. Rinsing in PBS for 20 minutes.
10. Progressive rinsing of slides from distilled water to xylene.
11. Mounting in Permount (Fisher Scientific Co. Fairlawn, NJ).

D. ANTIBODIES

Optimal dilutions of antibody (peroxidase method steps 3 and 5) were determined by using cross titrations on sections of human tonsil. The optimal dilutions, source, and specificity of Monoclonal Antibodies (McAbs) used in various studies is shown in Tables 2 and 3. The original terminology (OKT, Leu, etc.) has been used throughout this thesis instead of CD nomenclature (Pallesen and Plesner,

TABLE 2

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	**SPECIFICITY**
T Cell and Subsets	
OKT3 (CD3)	Pan T (Kung, et al, 1979).
OKT4 (CD4)	Helper T (Ledbetter, et al,1981).
OKT8 (CD8)	Suppressor T (Kung, et al, 1979).
Leu1 (CD5)	Pan T (Ledbetter, et al,1981).
Leu2A (CD8)	Suppressor T (Ledbetter, et al,1981).
Leu3A (CD4)	Helper T (Ledbetter, et al,1981).
Leu4 (CD3)	Pan T (Ledbetter, et al,1981).
Leu5 (CD2)	E receptor (Ledbetter, et al,1981).
T11 (CD2)	E receptor (Ip, et al, 1982; Greaves, et al, 1981).
OKT11A (CD2)	E receptor (Greaves, et al, 1981).
T101 (CD5)	Pan T, some B cell neoplasia (Royston, et al, 1980).
3A1 (CD7)	Suppressor T cells, subset of helper T cells (Cossman, et al, 1983), K cells (Herberman and Ortaldo, 1981).

TABLE 2 (Continued)

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	SPECIFICITY
Immaturity Antigens	
OKT6 (CD1a)	Immature (common) thymocytes (Kung, et al, 1980), epidermal dendritic cells (Chapter 4).
OKT9	Immature thymocytes, T cell ALL (Kung, et al, 1980), wide range of proliferating cell types in culture (Greaves,et al, 1981), eg., mammary carcinoma, teratocarcinoma. Transferrin receptor.
OKT10 (CD38)	Immature thymocytes (Kung, et al, 1980), bone marrow progenitor cells some activated T cells (Kung, et al, 1980), K cells (Herberman and Ortaldo, 1980).
Killer/Natural Killer	
HNK1(Leu7)	Granular lymphocytes with K/NK function (Abo and Balch, 1981),fetal hamster olfactory epithelium, (Ward, 1986) human neuroectodermal tumours, (Cailland,et al, 1984) neural and neuroectodermal cells of several species (Lipinski, et al,1983; Schuller-Petrovic, et al, 1983).
B Cell	
B1 (CD20)	B cells, B cell lymphomas (Nadler, et al, 1981a).
B2 (CD21)	Subset of B cells and B cell lymphomas (Nadler, et al, 1981b).
Leu14 (CD22)	B cells, weakly with monocytes, but not macrophages.
Kappa	Anti-kappa light chains.
Lambda	Anti-lambda light chains

TABLE 2 (CONTINUED)

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	**SPECIFICITY**
HLAD Locus	
HLADR	Monocytes, macrophages, Langerhans' cells, activated T cells, B cells (Warnke and Levy, 1980).
Monocyte/Macrophage/D Cell	
OKM1 (CD11)	Monocytes, macrophages, myeloid series (Breard, et al, 1980).
MO2 (CD14W)	Monocytes, macrophages (Todd, et al,1981).
LeuM1 (CD15)	Monocytes, macrophages, myeloid series (Hanjan, et al, 1982) adenocarcinomas, (Sheibani, et al, 1986), interdigitating reticulum cells, (Hofman, et al, 1984), Reed-Sternberg cells, (Pinkus, et al, 1985; Hsu, et al 1986).
LeuM3 (CD14)	Monocytes, macrophages (Dimitriu-Bona, et al, 1983).
My3	Monocytes (Civin, 1983).
Acid Alpha Naphthyl Acetate (Non-specific) Esterase	Macrophages ("Histiocytes") (Yam and Li, 1971)
R423	Dendritic reticulum (follicular dendritic) cells (Naiem, et al, 1983).
OKT6 (CD1a)	Epithelial dendritic cells (Chapter 4)
Non Lineage Restricted	
2D1 (CD45)	T200 Leukocyte common antigen (Pizzolo et al 1980).

Table 2 (Continued)

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	**SPECIFICITY**
Common Acute Leukaemia	
J5 (CD10)	Common acute lymphoblastic leukaemia antigen, (Ritz, et al, 1980), adult and fetal nonhaematopoietic tissue (Metzgar, et al, 1981) , immature T cells (Hsu, et al, 1985), neutrophils (Cossman, et al, 1983), germinal centre B cells, (Hsu, et al, 1984), B cell lymphomas and leukaemias (Hsu, et al, 1984; Cossman, et al, 1984).
Myeloblastic Leukaemia cell lines	
My10 (CD34)	Kg-1, Kg-1 a cell lines, some ALL and ANL, myeloid progenitor cells (Civin, et al, 1983, 1984).
My11 (CD45R)	Kg-1, Kg-1 a cell lines, monoblastic, B-lympho-blastoid lines, E rosette+ and E rosette- PBL, monocytes, some ALL, ANL, CLL, CFU-C but not CFU-E or BFU-E (Civin, et al, 1983, 1984).
My12	Kg-1, Kg-1a, U-937, K-562, HEL cell lines, some ANL, CML, ALL (Civin, et al, 1983, 1984).
My13	Kg-1, Kg-1 a cell lines blast cells from less than 10% of ANL or ALL, CLL. (Civin, 1983)
Leukaemia/Lymphoma	
Be1	Leukaemic CTCL lymphocytes, lymph nodes infiltrated by CTCL, Epstein Barr virus transformed cell lines and some long term T cell lines.
Be2	Peripheral blood lymphocytes from 75% of CTCL patients, Epstein Barr virus cell lines, some T cell lines and a subpopulation of lymphocytes from 5 of 8 patients with B cell chronic lymphocytic leukaemia

Table 2 (Continued)

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	**SPECIFICITY**
Control	
MOPC21	No known specificity.
Mouse Ascites	No known specificity.

NOTES OF EXPLANATION:

1. KG-1 is a myeloblastic leukaemia cell line derived from a patient with nonlymphocytic leukaemia (Koeffler, and Golde, 1978). The KG-1a cell line arose from it as a spontaneous tissue culture variant (Koeffler, et al., 1980). KG-1a cells are phenotypically less differentiated than KG-1 cells, and have the morphological and cytochemical features of primitive haematopoietic blast cells. (Koeffler, et al., 1980).

2. The McAbs MY10-13 have been raised against determinants on KG-1a cells. None of these antibodies react with blood granulocytes, red blood cells, or platelets from normal donors. Large subsets of peripheral blood lymphocytes and monocytes express the My11 antigen. My10, 12, 13 antigens are not expressed on significant numbers of blood lymphocytes or monocytes. My11 is found in 25% of bone marrowcells of normal donors. My10, 12, 13 bind only subpopulations of normal marrow cells.

3. Sources of McAbs:

A. Orthoimmunobiology. Raritan, New Jersey, USA: OKT3, 4, 6, 8, 9, 10, 11a, and OKM1.

B. Becton Dickinson Facs Systems Ltd., Sunnyvale, California, USA: Leu1, 2A, 3A, HLADR, LeuM1, LeuM3, Leu14, Anti-Kappa, and Anti-Lambda.

C. Coulter Ltd., Bethesda, Maryland, USA: B1, B2, T11, M02, and J5.

D. Hybritech, Inc., T101.

E. Barton Haynes, M.D., Dept. of Medicine, Duke University, North Carolina, USA: 3A1.

F. Toru Abo, Ph.D./Charles Balch, M.D. Departments of Microbiology and Surgery, University of Alabama, Birmingham, Alabama, USA: HNK1

G. David Mason, M.D., Department of Haematology, John Radcliffe Infirmary, Oxford, England: R423.

H. Curt Civin, M.D., Department of Paediatric Oncology, The John Hopkins Oncology Center, Baltimore, Maryland, USA: MY3, MY10-13, M.O.P.C. 21.

I. Carole Berger, Ph.D., Department of Dermatology, Columbia College of Physicians, New York, New York, Be1 and Be2.

J. American type Tissue Collection, Rockville, Maryland: M.O.P.C. 21. Mouse myeloma protein (culture supernatant with no known specificity, IgG1 Kappa produced by the P3 x63 AG B cell line.

4. ABBREVIATIONS

ALL: acute lymphoblastic leukaemia; CLL: chronic lymphocytic leukaemia; PBL: peripheral blood lymphocytes; BFU-E: burst forming units - erythroid; CFU-E: colony-forming units erythroid; CFU-C colony forming units - culture; ANL, acute nonlymphocytic leukaemia.

TABLE 3-DISTRIBUTION OF 33 MONOCLONAL DETERMINANTS IN HUMAN TONSIL

Antibody	No. of tonsils tested	Lymphoid follicles	Interfollicular areas	Crypt epithelium	Optimal dilution of primary antibody
Leuk 2D1	2	+++	+++	+	1:10
Leu4/5	2	++	+++	+	1:5
T11+OKT11A	6	+	+++	+	1:10
Leu1	15	+	+++	+	1:20
OKT3	15	+	+++	+	1:10
T101	6	+	+++	±	1:10
Leu3A	15	+	+++	+	1:10
3A1	8	+	+++	+	1:50
OKT4	15	+	+++	+	1:10
Leu2A	15	+	++	++	1:10
OKT8	15	+	++	++	1:10
B1	15	+++	+	-	1:10
B2	8	++	-	-	1:10
HNK1 (Leu7)	15	++	+	+	1:50
HLADR	15	+++	+	++	1:20
OKM1	5	+	+	-	1:10
MO2	5	++	±	-	1:10
MY3	5	+	-	-	1:10
LeuM3	5	++	+	-	1:20
LeuM1	5	-	+	-	1:20
R423	15	++	-	-	1:5
OKT6	15	-	-	++	1:10
OKT9	15	+++	+	-	1:10
OKT10	15	+	+	++	1:10
MY10	5	-	-	-	-
MY11	5	++	++	+	1:10
MY12	5	-	-	-	-
MY13	5	+	-	-	1:10
J5	8	±	-	-	-
Be1	2	-	-	-	-
Be2	2	-	+	-	1:50
MOPC21	5	-	-	-	-
Mouse ascites	3	-	-	-	-

++++, majority of cells stain positively; +, occasional positive cells; ++, intermediate between + and +++; ± equivocal reaction, -, negative

1987) for the sake of uniformity as this is consistent with the authour's previous publications and the CD agreement has come into force since most publications were written.

Because of limited funds and availability of various reagents and or tissue during the studies outlined in this thesis the whole range of McAbs mentioned in Table 2 was not tested in each specimen. The McAbs utilized in each chapter will therefore be outlined separately.

E. CONTROLS

Positive controls were carried out by testing sections of tonsil or thymus (once the pattern of staining in this tissue was ascertained).

Negative controls consisted of:

1. Omission of primary antibody.
2. Omission of primary and secondary antibody; i.e., application of DAB alone.
3. Use of an irrelevant primary antibody; eg., application of MOPC 21 (antibody with no known specificity).
4. Use of mouse ascites as primary reagent.
5. Substitution of the second layer antibody with peroxidase conjugated goat antirabbit IgG (Tago, Inc.).
6. The use of a battery of antibodies directed against several specificities also served as an internal control.

An endogenous peroxidase blocker was not used as methanol - HC1-H202 mixture was found to inhibit the staining reaction of non-neoplastic lymphoid cells (McMillan, et al., 1982a). However, it was considered that the control utilizing DAB alone would detect any endogenous peroxidase activity in elements such as red cells.

The possibility that the sodium azide preservative incorporated in the monoclonal reagents might significantly inhibit the immunoperoxidase reaction (Dixon and Webb, 1964) was tested using monoclonal T101, (Table 2) which is obtainable in vials with and without the presence of sodium azide. A series of sections of human tonsil and skin

(infiltrated with lichen planus) were tested using T101 as primary antibody with and without sodium azide. The quality and intensity of staining was marginally better in the tissues treated with antibody plus azide.

F. QUANTIFICATION

An assessment of the proportion of histiocytic and lymphoid cells expressing various determinants was obtained by counterstaining of sections with hematoxylin (with or without eosin) or methyl green. A minimum of 300 infiltrating cells (histiocytic plus lymphocytic) was counted per specimen by the observer (E.M.).

Photomicrographs were taken with a Zeiss photomicroscope. Positive peroxidase staining is brown in counterstained and non-counterstained specimens. In non-counterstained specimens with green filter interposed (to enhance contrast) positive staining is black against a green background. A green filter was used to permit better black and white reproduction for journals.

G. ALPHA-NAPHTHYL ACETATE ESTERASE STAIN

Esterase cytochemical identification of histiocytes was performed by the method of Yam and Li (1971).

Sections were placed in an incubation medium consisting of 1.6 l of hexazotised pararosaniline, 20 ml of 0.1 M sodium cacodylate buffer and 0.5 ml of 1% alpha-naphthyl acetate adjusted to pH 6.3.

The slides were incubated at 37 degrees centigrade for 60 minutes, washed in running tap water for 10 minutes and counterstained in 1% methyl green. The counterstained slides were then washed for one minute in distilled water, dehydrated through graded alcohols and cleared with synthetic medium.

Additional pertinent description of methods will be given in each chapter.

CHAPTER FOUR

I. MAPPING OF DETERMINANTS FOUND ON NORMAL LYMPHORETICULAR, LYMPHOMA, AND MYELOBLASTIC LEUKAEMIA CELLS IN NON-MALIGNANT LYMPHOID TISSUE AND SKIN.

Including:

A. Introduction
B. Materials and Methods
C. Results
   Tonsil
   Lymph Node
   Thymus
   Skin
   Esterase Staining.
D. Discussion
   Tonsil and Lymph Node
   Thymus
   Skin

II. POSITIVE PATCH TESTS IN ALLERGIC CONTACT DERMATITIS

Including:

A. Introduction
B. Materials and Methods
C. Results
D. Discussion.

CHAPTER FOUR

I. MAPPING OF DETERMINANTS FOUND ON NORMAL LYMPHORETICULAR (T, B, K IMMATURE AND MACROPHAGE), LYMPHOMA, AND MYELOBLASTIC LEUKAEMIA CELLS IN NON-MALIGNANT LYMPHOID TISSUE (TONSIL, THYMUS, LYMPH NODE) AND SKIN.

A. INTRODUCTION

Prior to this study, the distribution in human tonsil of T cells and B cells has been documented in experiments using T cell heteroantisera (Lamelin, et al., 1978) and immunoglobulin antisera (Curran and Jones, 1977). The recent availability of monoclonal antibodies (Kohler and Milstein, 1975) with their improved specificity and broad range of determinants detected (including T subsets) (Kung, et al., 1979; Kung, et al., 1980; Ledbetter, et al., 1981) and the dearth of studies using these reagents in "normal" tissues suggests that studies using these reagents would be worthwhile. The establishment of the distribution of these determinants is important for several reasons:

1) Malignant lymphoid cells may retain sufficient similarity to their normal non-malignant counterparts so that their selective metastatic pattern may be similar to normal migration pathways (McMillan, 1973; Baird, 1978). Lymphoreticular neoplasia are unusual in that they are derived from cells whose normal capacity includes migration through various body compartments. Study of normal lymphoreticular topography may, therefore, aid our understanding of metastasis.

2) Physiologic knowledge of potential areas of interaction of lymphocyte subpopulations, eg. T and B cells, may be obtained.

3) Because non-tumour specific monoclonal antibodies may be used therapeutically [eg. Leu 1 in CTCL, (Miller and Levy, 1981)] knowledge of the distribution and prevalence of the determinants involved might facilitate the prediction of possible side-effects of monoclonal antibody therapy. This

is important, since knowledge of the normal tissue distribution of various determinants is limited. The accidental finding of the OKT6 (immature thymocyte) determinant on epidermal dendritic cells, (Chapter 5) in parallel studies outlines this. McAbs have also recently been raised to myeloblastic leukaemia cells (Civin, et al., 1983, 1984) Their reactivity with normal lymphoid tissue needs to be tested for the same reasons.

4) The establishment of the regular distribution of a particular determinant in relatively easily procured tissue, eg. tonsil, would provide a useful control when immunophenotyping lymphomas.

B. MATERIALS AND METHODS

The antibodies used are shown in Table 3 and their known specificities at the time of study are shown in Table 2. The procedure and negative controls are as outlined elsewhere(Chapter 3). Cryostat sections of specimens of human tonsil obtained from subjects 5 to 25 years of age were studied. The number of tonsils tested with each antibody is given in Table 3. Specimens of lymph node were obtained incidentally from five adults undergoing vascular surgery. One specimen of neonatal thymus was obtained from a patient undergoing thoracic surgery. Five specimens of normal skin were procured from volunteers undergoing hair transplant surgery.

The following antibodies were tested in each lymphoid organ:

A. Tonsil: All McAbs.

B. Lymph node: OKT3, OKT4, OKT8, OKT6.

C. Thymus: OKT3/Leu1/T11 (pan T); OKT8/Leu2A (suppressor T); OKT4/Leu3A (helper T); OKT6 (common thymocytes); OKT9/OKT10 (immature thymocytes).

D. Skin: B1, B2, T11, OKT11A, 3A1, T101, HNK1(Leu7), OKT9, T10, HLADR, J5, Leu M3, Leu1, Leu2A, Leu3A, OKT3, OKT4, OKT8, OKT6, LeuM1, R423, My3, My10, My11, My12, My13,

Be1, Be2, MOPC21, Mouse Ascites.

The presence of macrophages was also tested in tonsil, lymph node, and thymus and skin by esterase cytochemistry (Chapter 3).

C. RESULTS:

TONSIL

The results obtained and the distribution of the determinants are shown in Table 3 and Figure 1. The antileukocyte antibody, Leuk.2D1, stains the majority of cells in lymphoid follicles and interfollicular areas. Certain determinants, however, have a discrete localization. The antigens detected by T11, OKT11A, Leu1, OKT3, T101, Leu3A, 3A1, OKT4, Leu2A and OKT8 have similar distributions with most reactivity in interfollicular areas (IFAs) (Plates 1 and 2) and occasional positive staining cells in lymphoid follicles (LFs) (Plate 3) and crypt epithelium (CE). Positive staining with T and T subset monoclonals in L.F.s is seen both in mantle zones (MZs) and in germinal centres (GCs) of secondary follicles (Plate 4). Leu1+, OKT3+, Leu3A+, 3A1+ and OKT4+ cells sometimes occur in dense clusters in GCs (Plate 5) or in a crescentic pattern (Plate 6) at the junction of the GC and MZ. Results with Leu2A/OKT8 are interesting in that, although the localization is predominantly in interfollicular areas as with other reagents, the number of cells staining positively is clearly fewer in number than with the other T cell antibodies tested. The OKT4 Leu3A (helper)/OKT8 Leu2A (suppressor) ratio is greater than or equal to 2:1 in all tonsils examined (Plates 7 and 8). The crescentic pattern frequently noted with Pan T and helper T cell antibodies is not evident with the suppressor cell reagents. It is not known if this reflects a true difference in the topographic distribution of helper and suppressor subsets, or whether the absence of the crescentic pattern is merely due to the overall smaller number of suppressor cells present, making

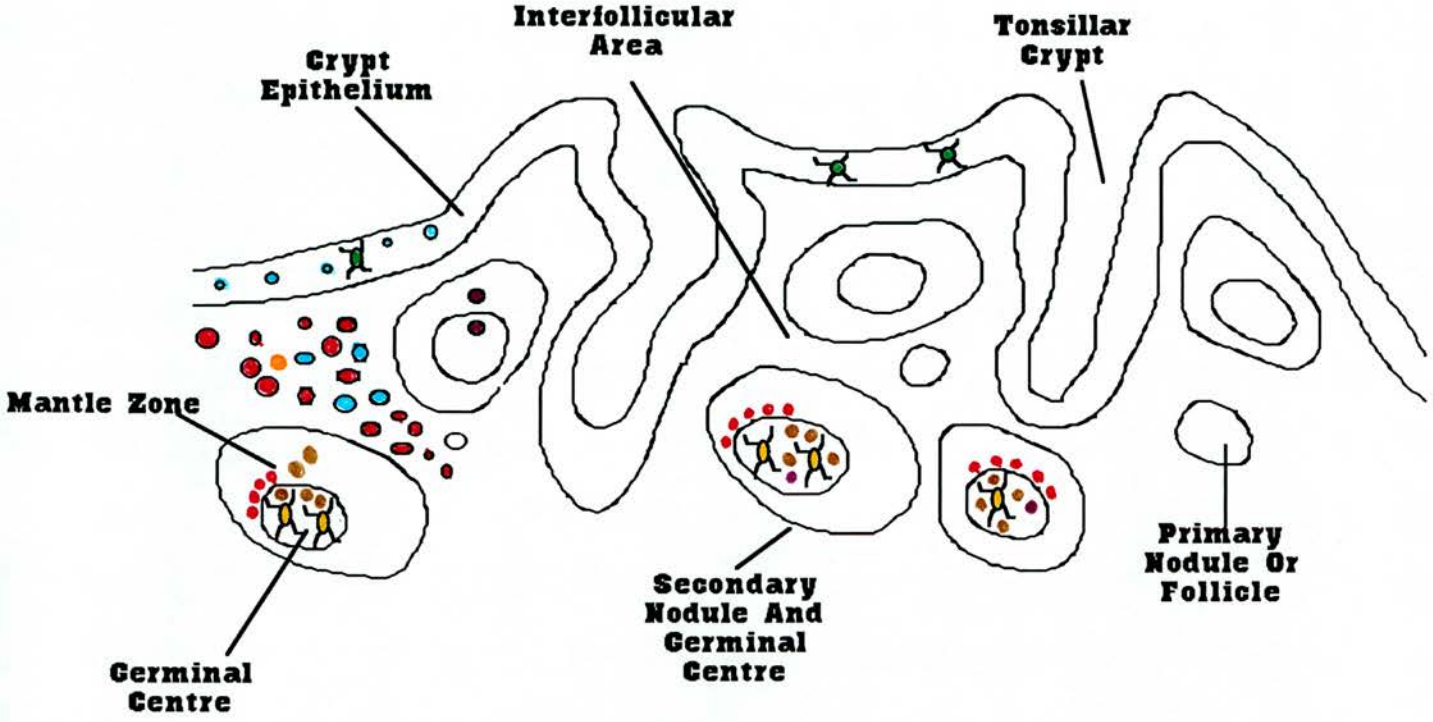

Figure 1. **Lymphoid Tissue Of Human Tonsil. Schematic Representation Of Distribution Of Monoclonal Determinants.**

- Pan T Cell (T11, OKT11a, Leu1, OKT3, T101)
- T Helper (OKT4, Leu3a)
- T Cytotoxic/Suppressor (OKT8, Leu2a)
- B Cell (B1, B2)
- Intrafollicular MPS (Mo2, My3, My13, R423, LeuM3, OKT9)
- Crypt Epithelial MPS (OKT6)
- Interfollicular MPS (Leu M1)
- K/Nk (HNK1)
- Be2 Leukaemia Lymphoma Antigen
- Negative Reaction: J5 (Common Acute Lymphoblastic Leukaemia) My10, My12, (Myeloid Leukaemia) Be1 Leukaemia/Lymphoma Antigen

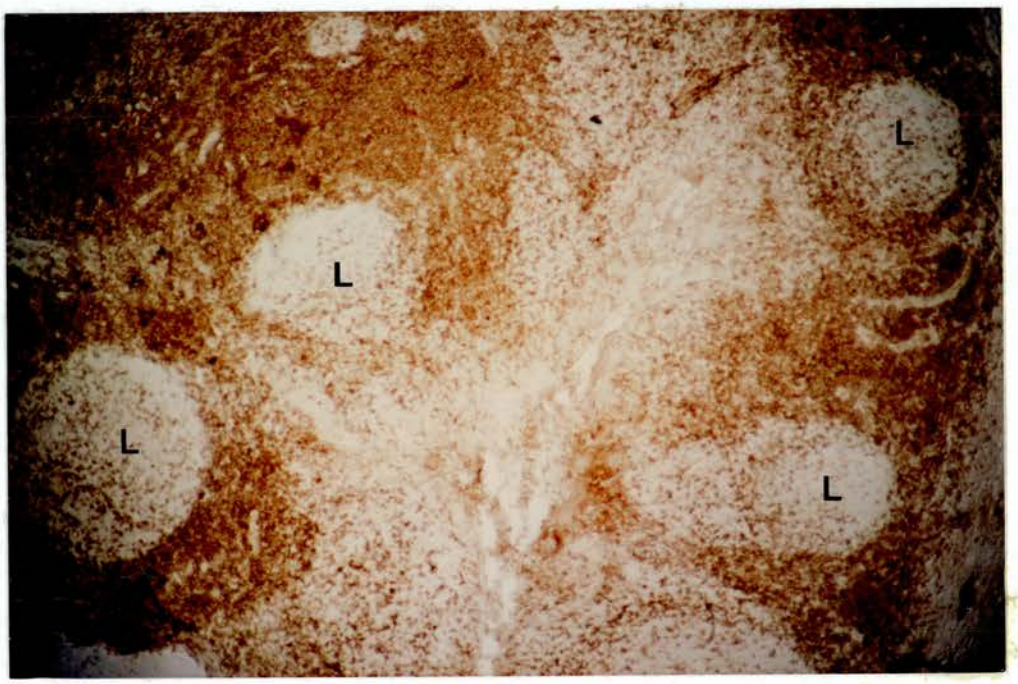

PLATE 1. **HUMAN TONSIL.** Preponderantly interfollicular distribution of Leu1+ (Mature T) lymphoid cells. Indirect immunoperoxidase. Non counterstained x 10. L = Lymphoid Follice I = Interfollicular Area

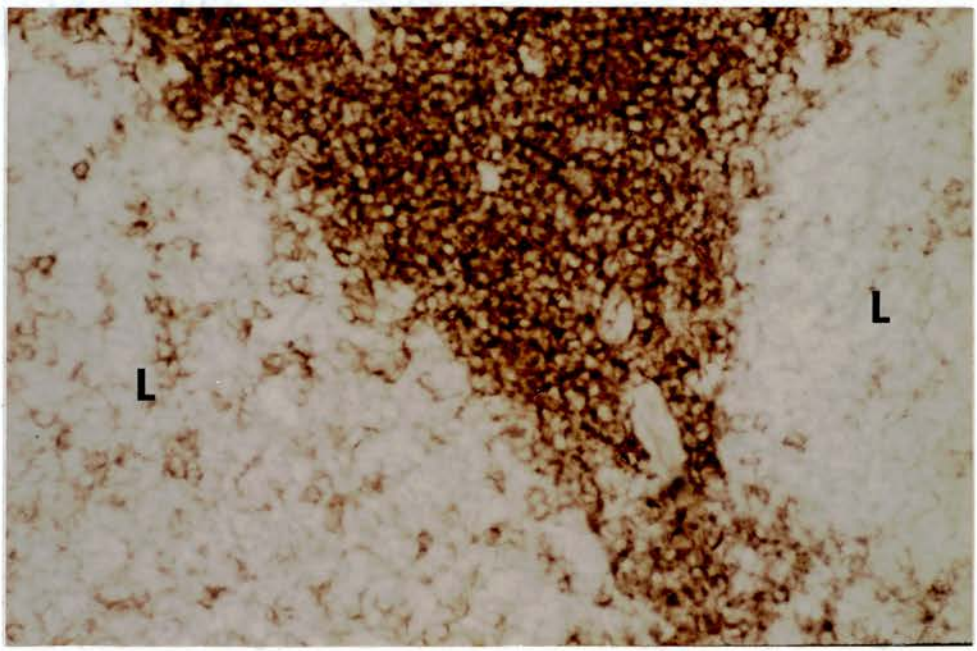

PLATE 2. **HUMAN TONSIL.** Leu 1+ interfollicular lymphoid cells with preponderantly negatively staining lymphoid follicles (L) adjacent. Indirect imunoperoxidase. Non counterstained x 64.

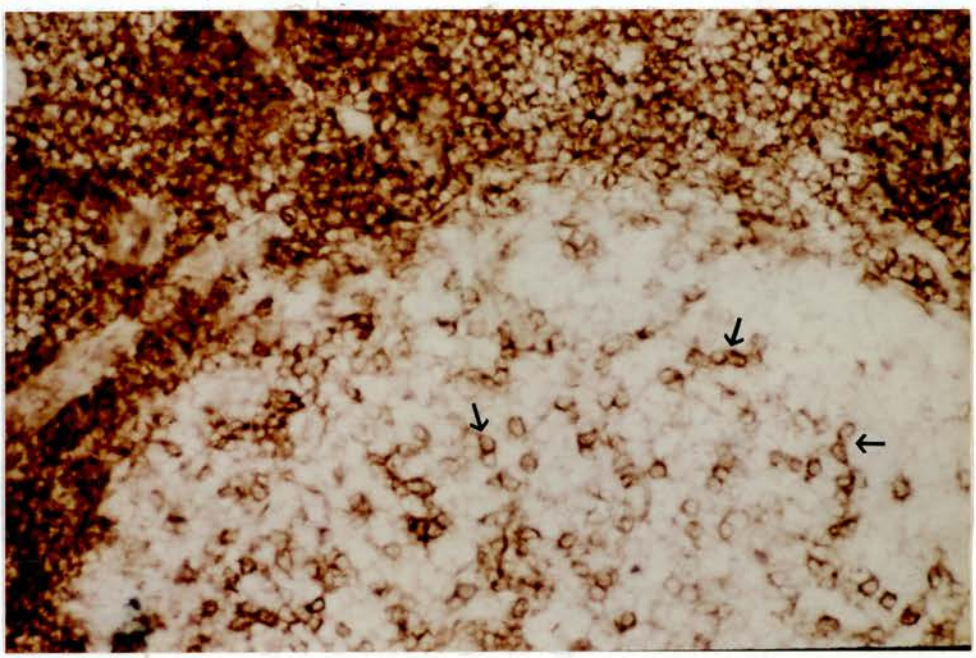

PLATE 3. **HUMAN TONSIL.** Intrafollicular Leu1+ lymphoid cells (arrow). Interfollicular area (upper quarter of field) contains numerous Leu1+ cells. Indirect immunoperoxidase. Non counterstained x 64.

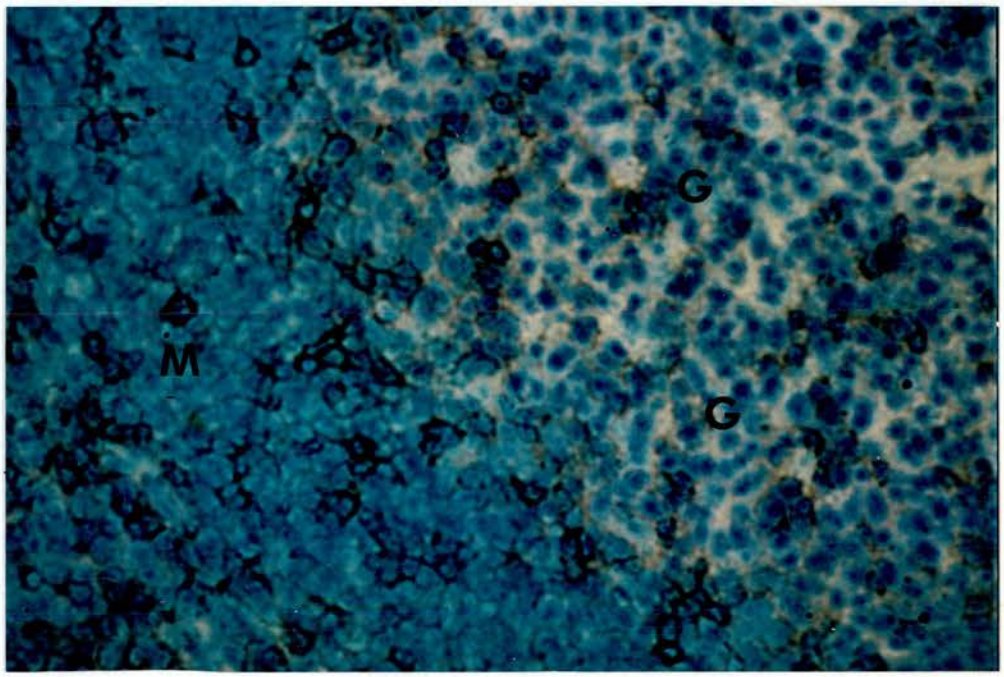

PLATE 4. **HUMAN TONSIL.** T11+ (E receptor antibody) lymphoid cells in germinal centre (G) and mantle zone (M) of secondary lymphoid follicle. Indirect immunoperoxidase. Counterstain haematoxylin x 100.

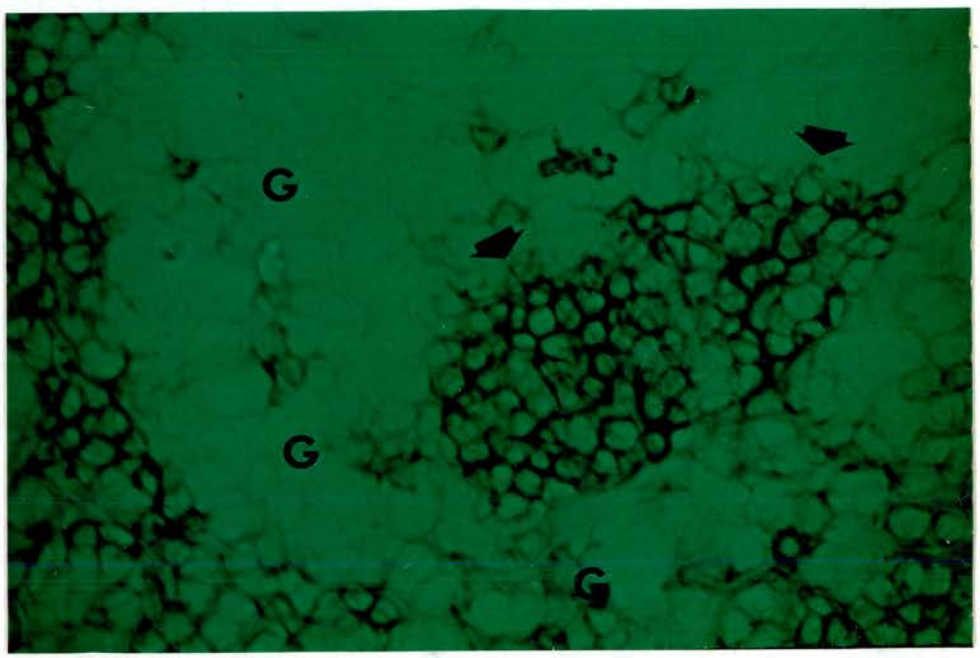

PLATE 5. **HUMAN TONSIL.** Cluster of 3A1+ cells (arrow) in germinal centre (G) of lymphoid follicle which mainly stains negatively with 3A1. Variable density of 3A1 staining suggest this antibody may react with intrafollicular dendritic cells in addition to lymphocytes. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

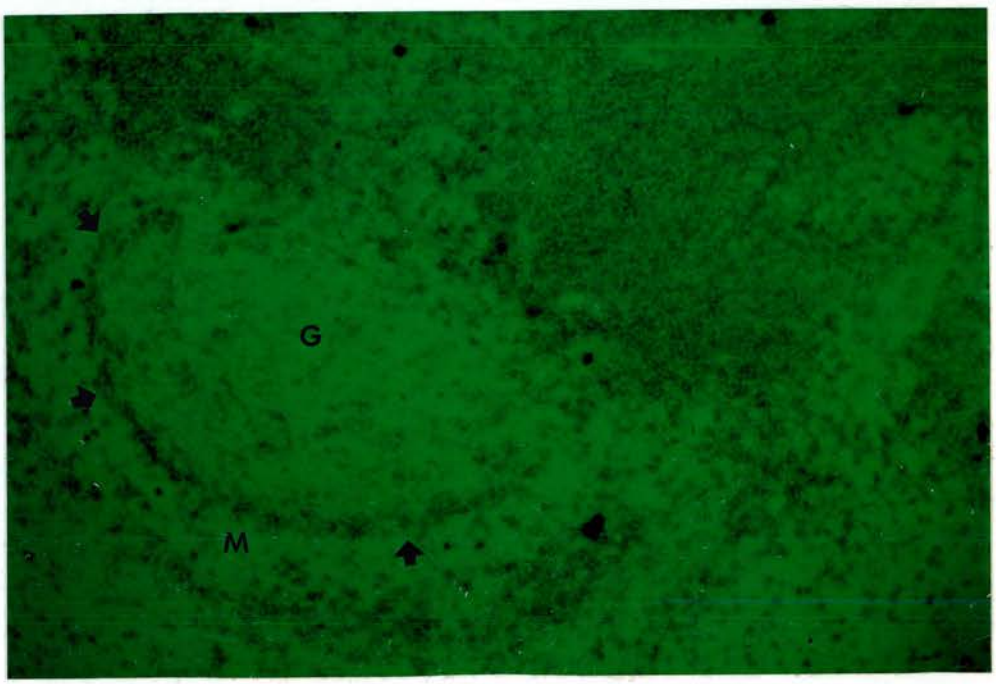

PLATE 6. **HUMAN TONSIL.** OKT4+ cells in crescentic pattern (arrow) at junction of germinal centre (G) and mantle zone (M) of secondary lymphoid follicle. Similar pattern is obtained with Leu3A. Indirect immunoperoxidase. Non counterstained. Green filter x 25.

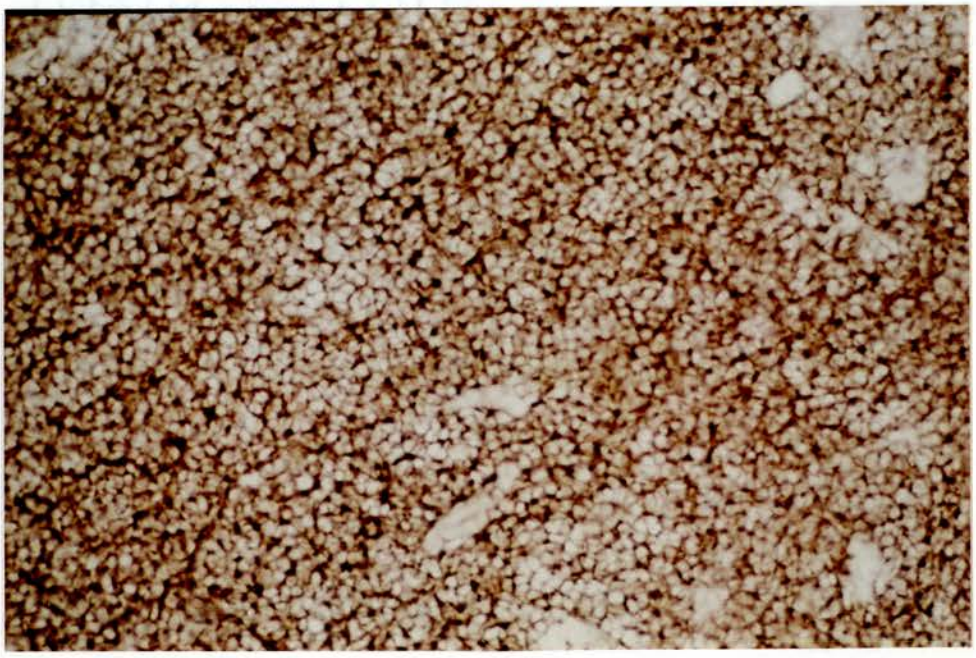

PLATE 7. **HUMAN TONSIL.** Interfollicular area Leu3A (helper T cell antibody) stains majority of cells. Indirect immunoperoxidase. Non counterstained x 64.

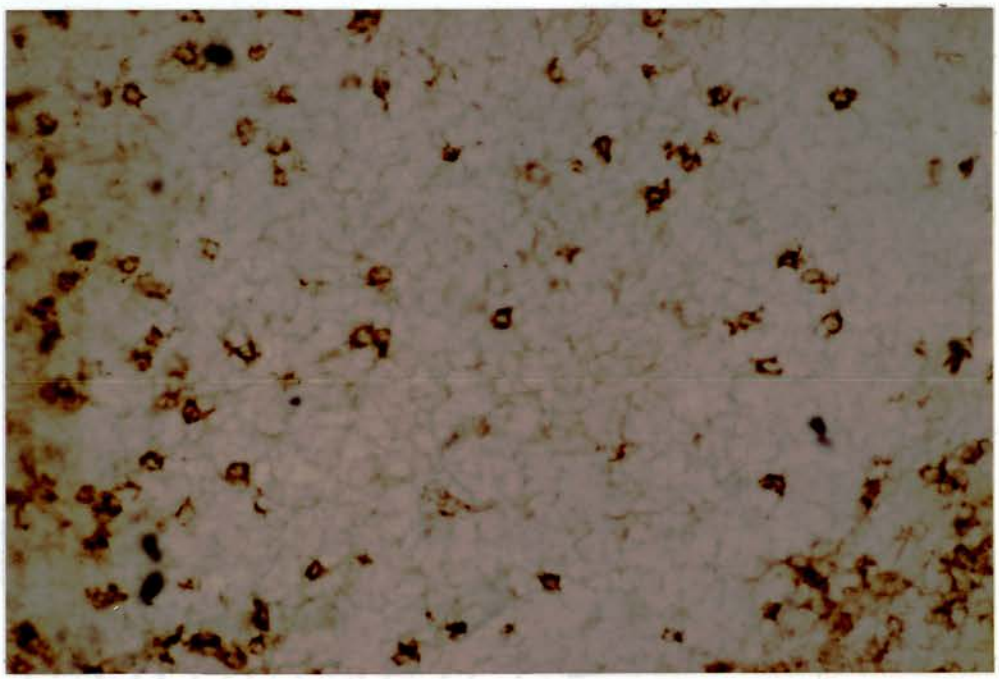

PLATE 8. **HUMAN TONSIL.** Interfollicular area. Leu2A (suppressor T cell antibody) stains minority of cells. Cf. Plate 7. Indirect immunoperoxidase. Non counterstained x 64.

discernment of this pattern difficult.

Leu 2A+/OKT8+ cells are more evident in crypt epithelium than Leu 3A+ (OKT4+ cells).

B1 and B2 reactivity is mainly within LFs (Plate 9) with occasional positive staining in IFAs and a negative reaction in crypt epithelium.

B1 results in membranous staining of most cells in LFs including the MZ of secondary follicles. Variable cytoplasmic and intercellular staining is often present in the germinal centre (GC). B2 produces staining of GCs and MZs with staining being more intense in the GCs. Cytoplasmic and intercellular staining is also present in GCs. In areas where negative staining cells permit better contrast of the B2 positive cells, a dendritic pattern is also observed in the GC with B2, suggesting that this antibody may also react with dendritic reticulum (follicular dendritic) cells.

The main localization of HNK1 cells is in LF's. (Plates 10 and 11) In secondary LFs they are mainly observed in the GCs but also occur in MZs.

Monoclonal HLADR stains most cells in LFs, occasional cells in IFAs and a dendritic population in CE. The HLADR staining in GCs and MZs in LFs is mainly peripheral, although cytoplasmic and intercellular staining is also seen in GCs. In the IFAs rounded staining is present, and in some areas a dendritic staining pattern is present.

The monocyte-macrophage monoclonals produce variable positive reactions. OKM1+ cells are occasionally observed in LFs and IFAs. OKM1+ staining often has a dendritic pattern in LFs. MO2+ and LeuM3+ cells are found in LFs. LeuM1+ cells are occasionally found in IFAs. R423+ cells are confined to LFs.

Monoclonal antibody R423 produces dense staining of the lymphoid follicles (Plate 12), and a dendritic staining pattern is often most evident at the edge of the positively

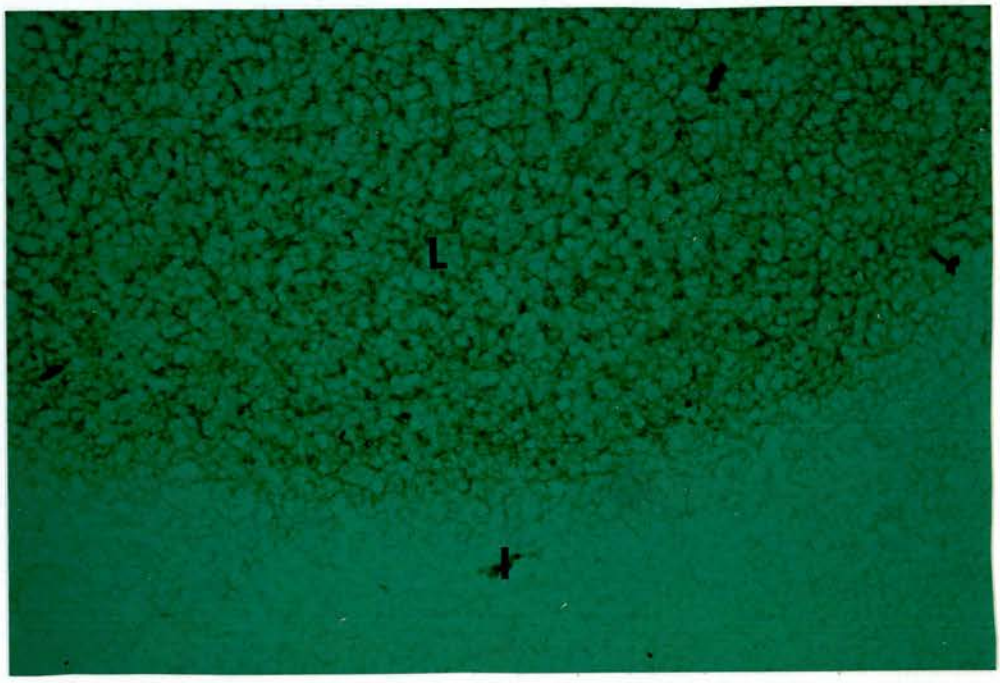

PLATE 9. **HUMAN TONSIL.** B2+ cells within lymphoid follicle (L). Interfollicular area (I) stains negatively. Indirect immunoperoxidase. Non counterstained. Green filter x 40.

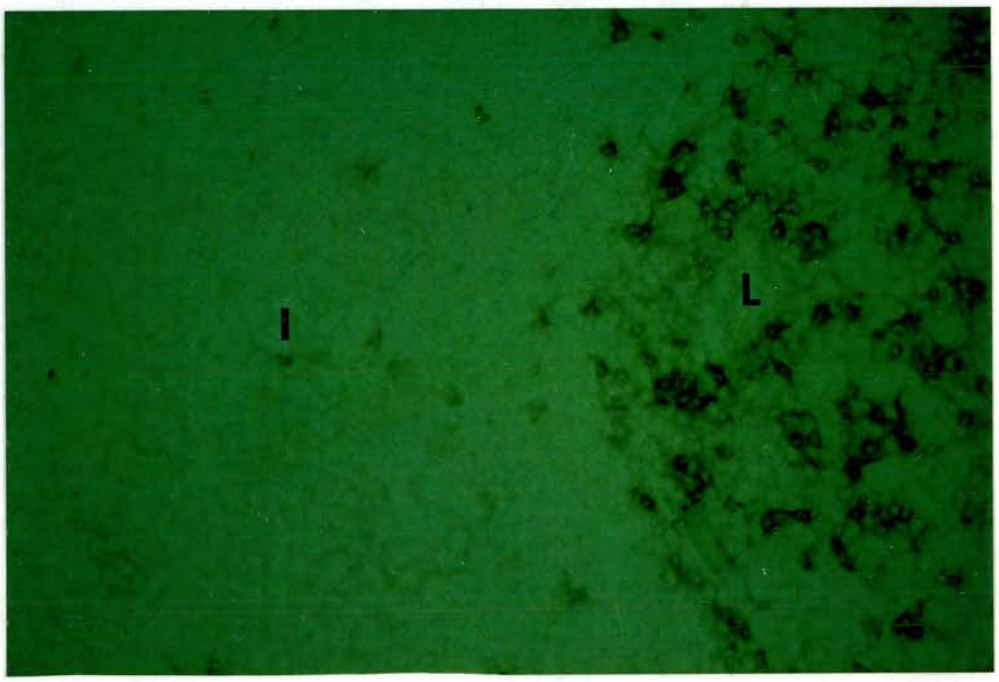

PLATE 10. **HUMAN TONSIL.** HNK1+ (Killer/Natural Killer) lymphoid cells mainly reside within lymphoid follicle (L) (right half of field) with interfollicular area (I) (left half of field) staining negatively Indirect immunoperoxidase. Non counterstained. Green filter x 100.

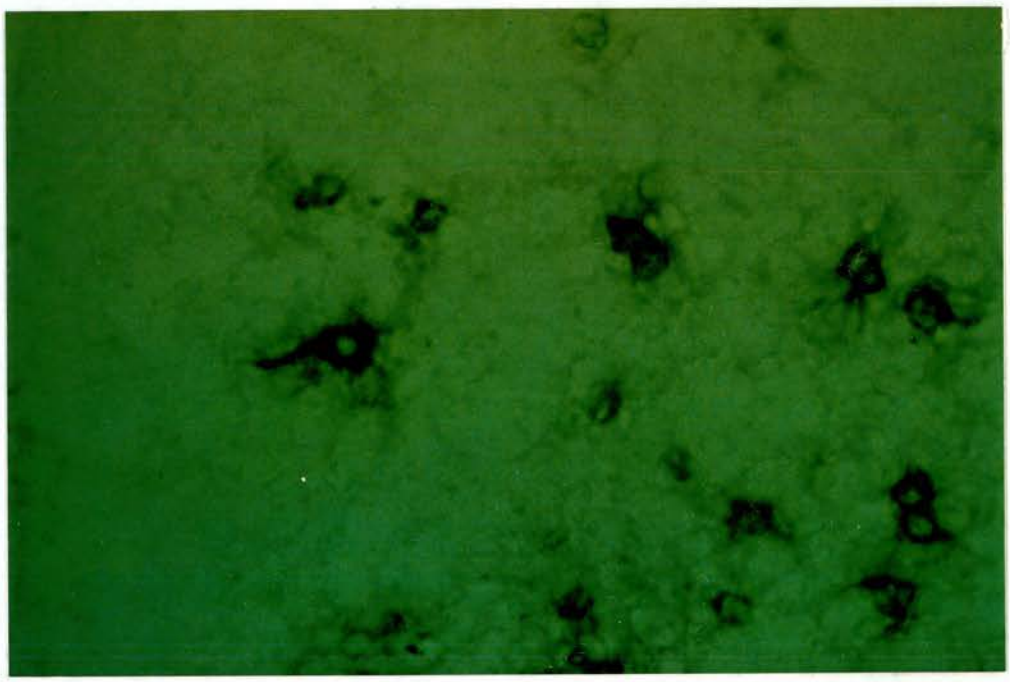

PLATE 11. **HUMAN TONSIL.** HNK1+ lymphoid cells (high power). Majority of positively staining cells show rounded staining pattern. However, cell left of centre exhibits dendritic staining. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

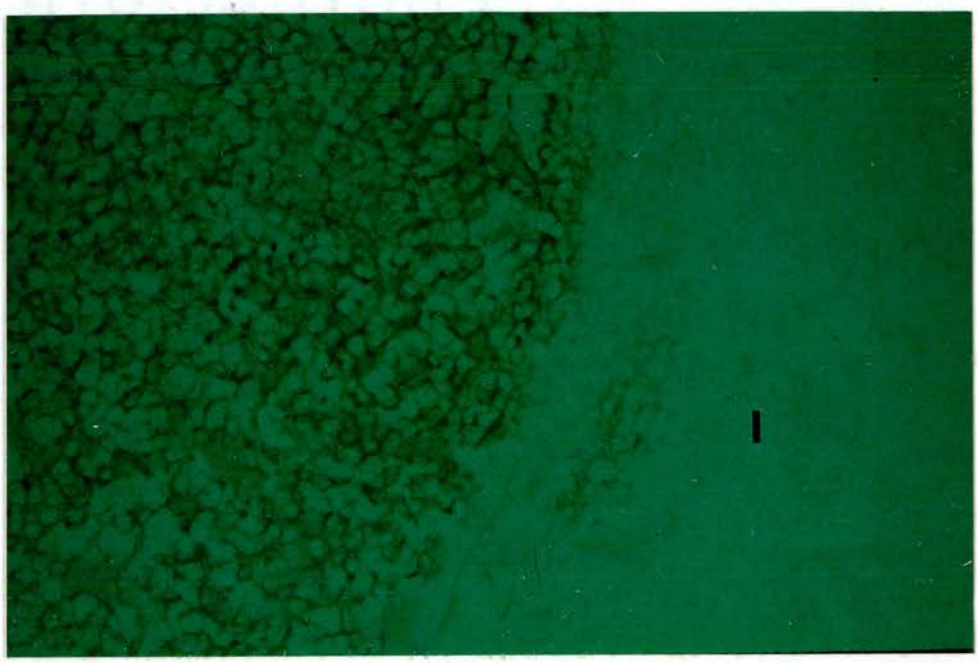

PLATE 12. HUMAN TONSIL. R423+ follicular dendritic cells (left half of field). Adjacent interfollicular area (I) (right half of field) shows predominantly negative staining pattern. Indirect immunoperoxidase. Non counterstained. Green filter x 64.

staining areas (Plate 13). Positively staining cells can therefore be seen in counterstained specimens extending into the MZ adjacent to GCs. Sometimes the dendritic pattern is evident in the densely staining GCs when surrounding negatively staining cells highlight the dendritic R423 cells.

The monocyte-macrophage monoclonals tend to produce membranous and cytoplasmic staining. The staining observed is most dense with optimal dilutions of LeuM1, LeuM3, and R423, when the monocyte-histiocyte monoclonals are compared.

Monoclonal OKT6 produces a similar reaction to HLADR in CE (Plates 14 and 15) but does not stain follicles or interfollicular areas.

No unequivocally positive cells are observed in any specimen with J5. However, diffuse poorly defined staining slightly greater than controls is often observed in LFs.

Monoclonal antibody OKT9 stains a large number of cells in LFs (Plate 16) and occasional cells in IFAs. Some of the OKT9+ cells in GCs are noted to be very large (Plate 16) and to show intense cytoplasmic staining.

OKT10 produces positively staining cells, mainly residing within LFs. A mixture of membranous and cytoplasmic stained cells is noted. Occasional clusters of OKT10+ cells are also noted adjacent to crypt epithelium. The cells mainly show intense cytoplasmic staining.

My10 and My12 produce a negative reaction (Table 3). My11 positivity is observed in a large number of cells in LFs and IFAs. My 13 stains dendritic macrophages in LFs.

Be1 produces a negative reaction whereas Be2 positively staining cells occur in small clusters in the interfollicular areas. The staining is weak.

MOPC21 and mouse ascites produce negative reactions.

LYMPH NODE (Figure 2)

The result in LFs and paracortex with OKT3, OKT4, OKT8 are similar in quantity and distribution to those obtained

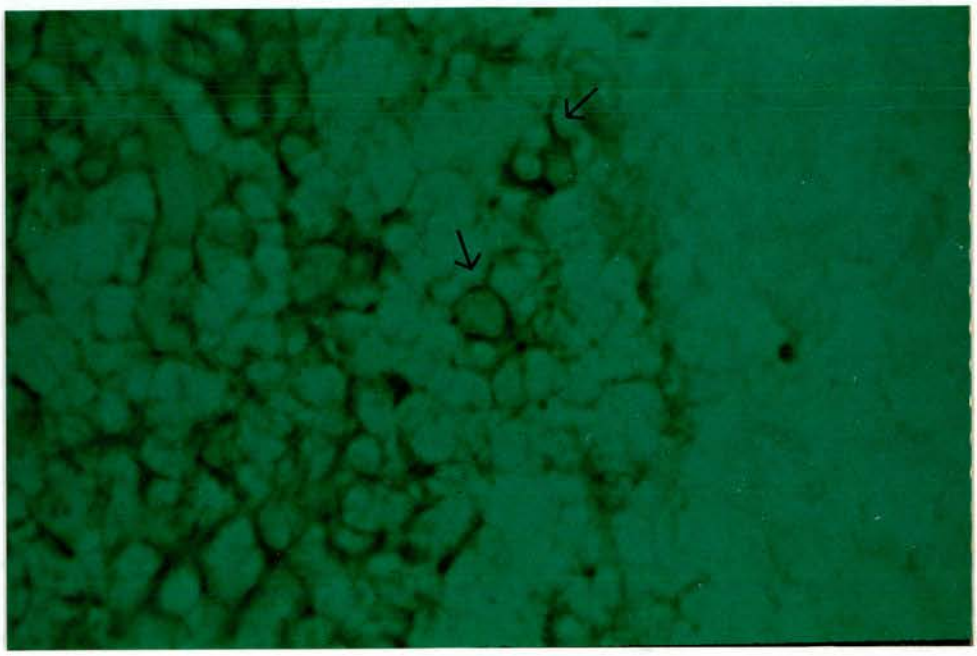

PLATE 13. **HUMAN TONSIL.** Dendritic nature of R423+ cells (arrow) evident at edge of lymphoid follicle. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

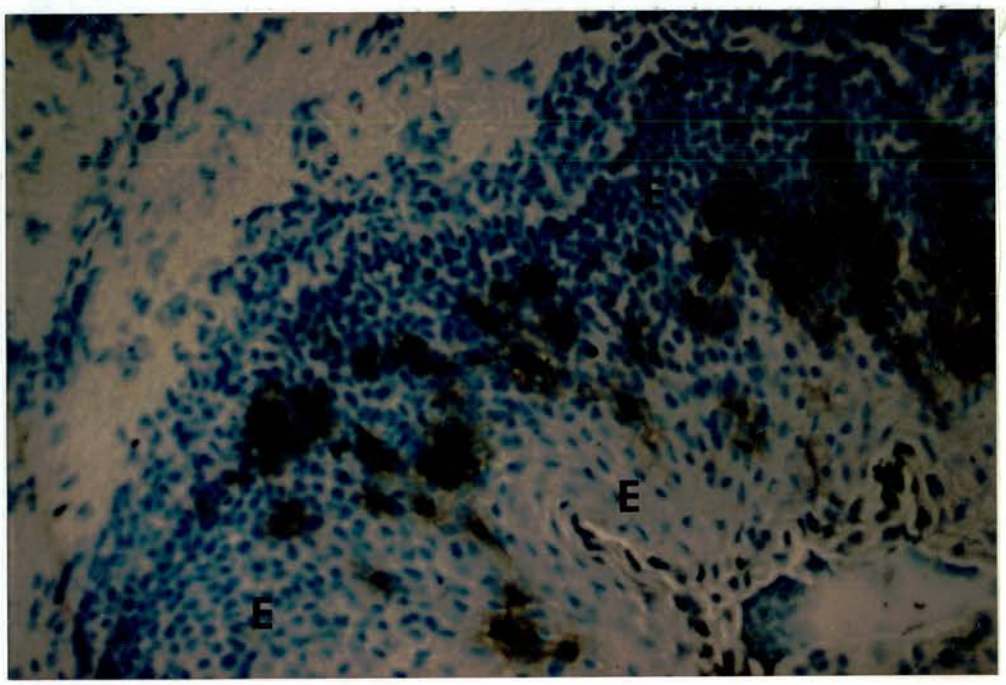

PLATE 14. **HUMAN TONSIL CRYPT EPITHELIUM.** OKT6+ intraepithelial dendritic cells at varous levels through epithelium (E). Indirect immunoperoxidase. Haematoxylin counterstain x 64.

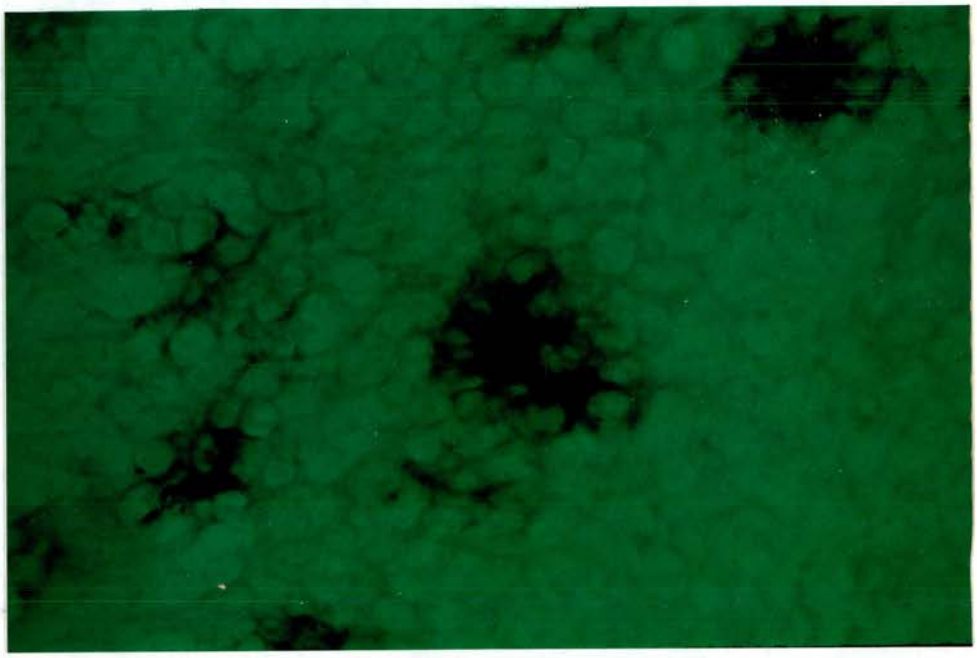

PLATE 15. **HUMAN TONSILLAR CRYPT EPITHELIUM.** Intraepithelial dendritic OKT6+ cells. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

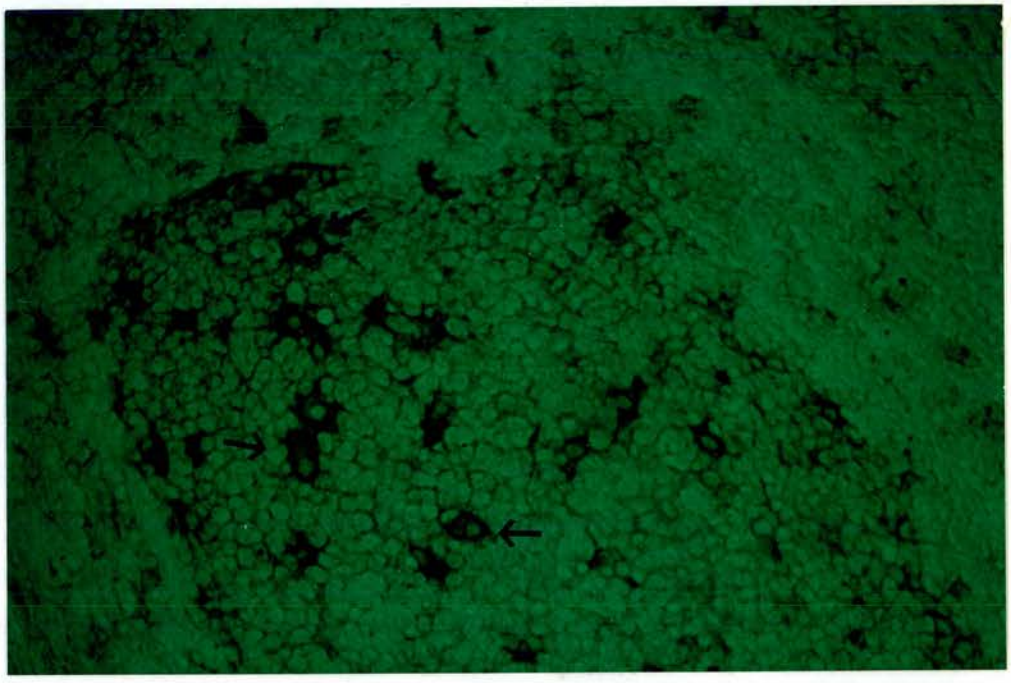

PLATE 16. **HUMAN TONSIL.** OKT9 produces staining of numerous cells in lymphoid follicle with dense staining of follicular macrophages (arrow). Indirect immunoperoxidase. Non counterstained. Green filter x 64.

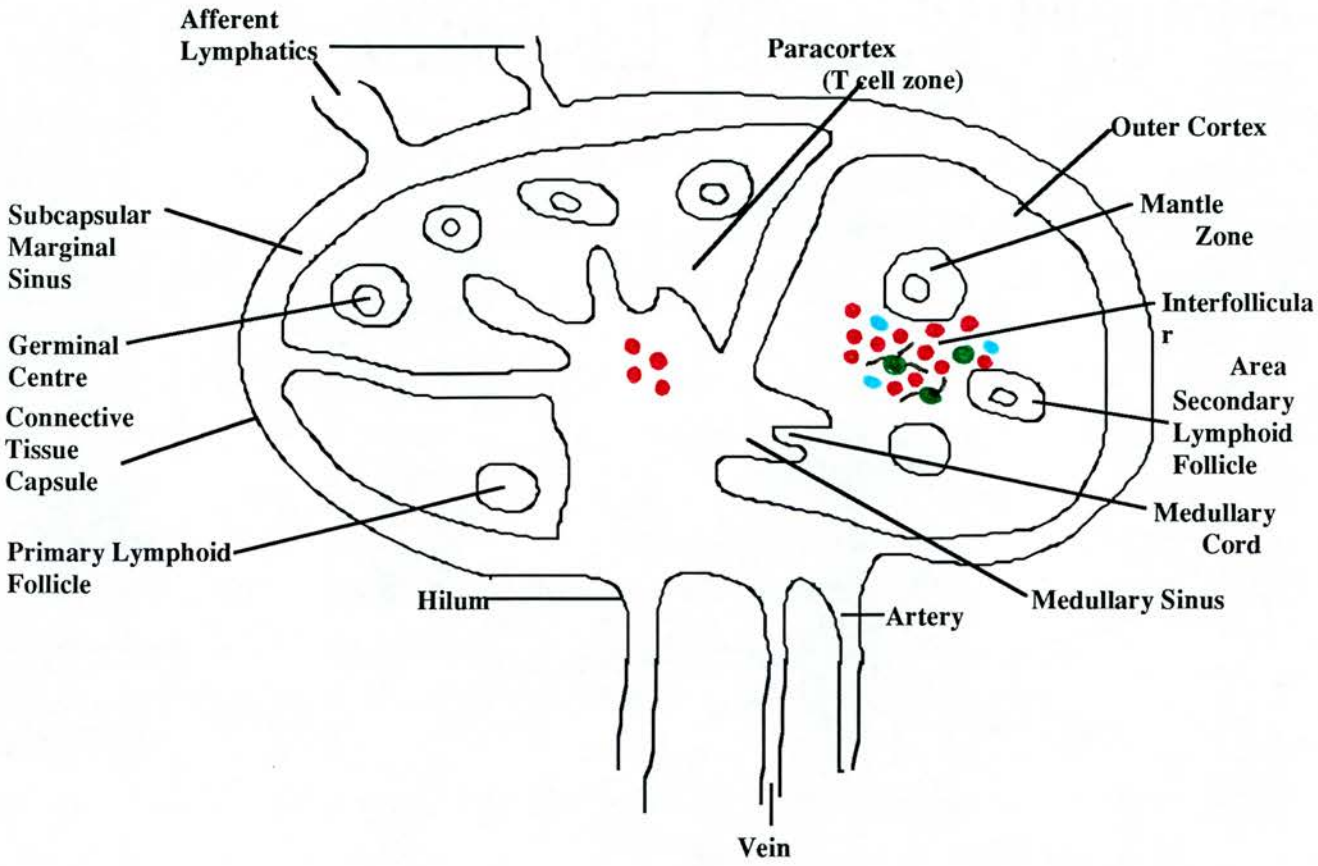

FIGURE 2. **Human Lymph Node. Schematic Representation Of Distribution Of Monoclonal Determinants.**

KEY

PAN T cell (OKT3)

T Helper (OKT4)

T Cytotoxic Suppressor (OKT8)

OKT6

Negative Reaction with Be1, Be2, (Leukaemia/Lymphoma)

in tonsillar LFs and IFAs. In addition, lymph node medulla shows variable staining with OKT3 (less than or equal to 50% of cells), the majority of these being OKT4+.

The results with OKT6 contrast with those in tonsil (where all cases show negative staining in the IFA for OKT6). Positively staining cells are identified in two of the five lymph nodes examined. They are located in localized clusters in the interfollicular areas (Plate 17) and also tend to produce a dendritic staining pattern. These clusters are only occasionally found, however, the majority of IFA producing a negative staining pattern. No positively staining cells are identified in primary or secondary follicles. The large number of cells staining positively with the mature T cell antibodies, especially with OKT3 and OKT4 which react with the majority of cells in the IFAs, make it impossible to determine whether this small population of OKT6 positive cells might also be expressing these markers. A dendritic staining pattern is not noted with any of these antibodies, however, making this less likely.

Be1 and Be2 produce negative reactions.

THYMUS

The results obtained in thymus are summarized in Table 4 and Figure 3. The antibodies are listed in order of their preferential reactivity with more primitive ontogenetic areas of the thymus (i.e. subcapsular region, cortex) through to the medulla where more mature T cells are thought to reside prior to their release into the bloodstream (Cantor, 1976; Stutman, 1978; Weissman, 1967).

SKIN (Figure 4)

Negative reactions occur with antibodies directed against B cells (B1, B2), and mature T cell/ T cell subsets (T11, OKT11A, 3A1, T101, Leu1, Leu2A, Leu3A, OKT3, OKT4, OKT8), K/NK (HNK1), two of three immature T cell (OKT9,

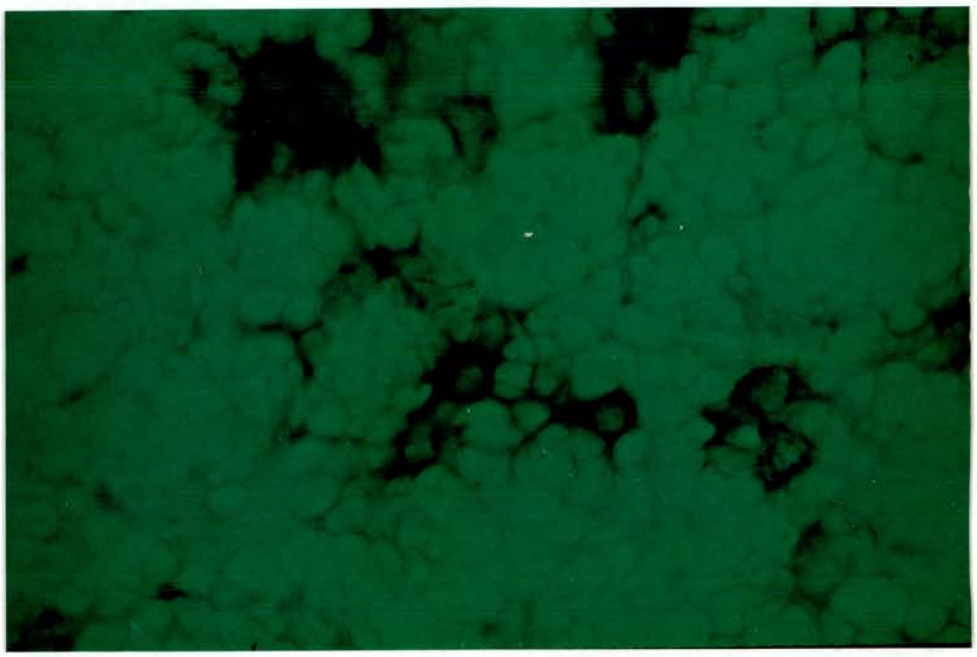

PLATE 17. **HUMAN LYMPH NODE.** OKT6+ dendritic cells occupying interfollicular area. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

TABLE 4. DISTRIBUTION OF T CELL SUBSETS IN THYMUS AND PERIPHERAL LYMPHOID TISSUE

Antibody	Thymus Subcapsular	Thymus Cortical	Thymus Medullary	Peripheral Lymphoid Tissue T Cell Zone (Interfollicular Areas and Lymph Node Paracortex)
OKT9	Majority of cells >70%	Minority of cells (=10%) Occasional OKT9+ clusters	Few cells (<10%)	Few cells (<10%)
OKT10	Majority of cells.70%	Majority of cells >70%	Few cells (<10%)	Few cells (<10%)
OKT6	Majority of cells >70%	Majority of cells >70% Decreasing strength of staining towards medulla	Few cells (<20%)	Few cells in 2 of 5 Lymph nodes; absent in IFA of tonsil
Leu1	Weak staining of cells (50%)	Majority of cells >70%	Majority of cells >70%	Majority of cells >70%
OKT3	Weak staining of cells (<10%)	Minority of cells <50% Variable strength. Occasional clusters of intensely staining cells	Majority of cells >70% Staining more intense than cortex	Majority of cells >70% Staining strength similar to medullory thymocytes
Leu2A/OKT8	Variable staining Minority of cells <50%	Majority of cells >70%	Approximately 30% of cells	Approximately 30% of cells
Leu3A/OKT4	Variable staining Minority of cells <50%	Majority of cells >70%	Approximately 60% of cells	Approximately 60% of cells
*R423	Negative	Negative	Negative	Negative (B Cell zone, lymphoid follicles, positive)

Note: R423* which stains dendritic cells in B cell zones of peripheral lymphoid tissue is included for comparison with OKT6 which stains dendritic cells associated with T cell populations.

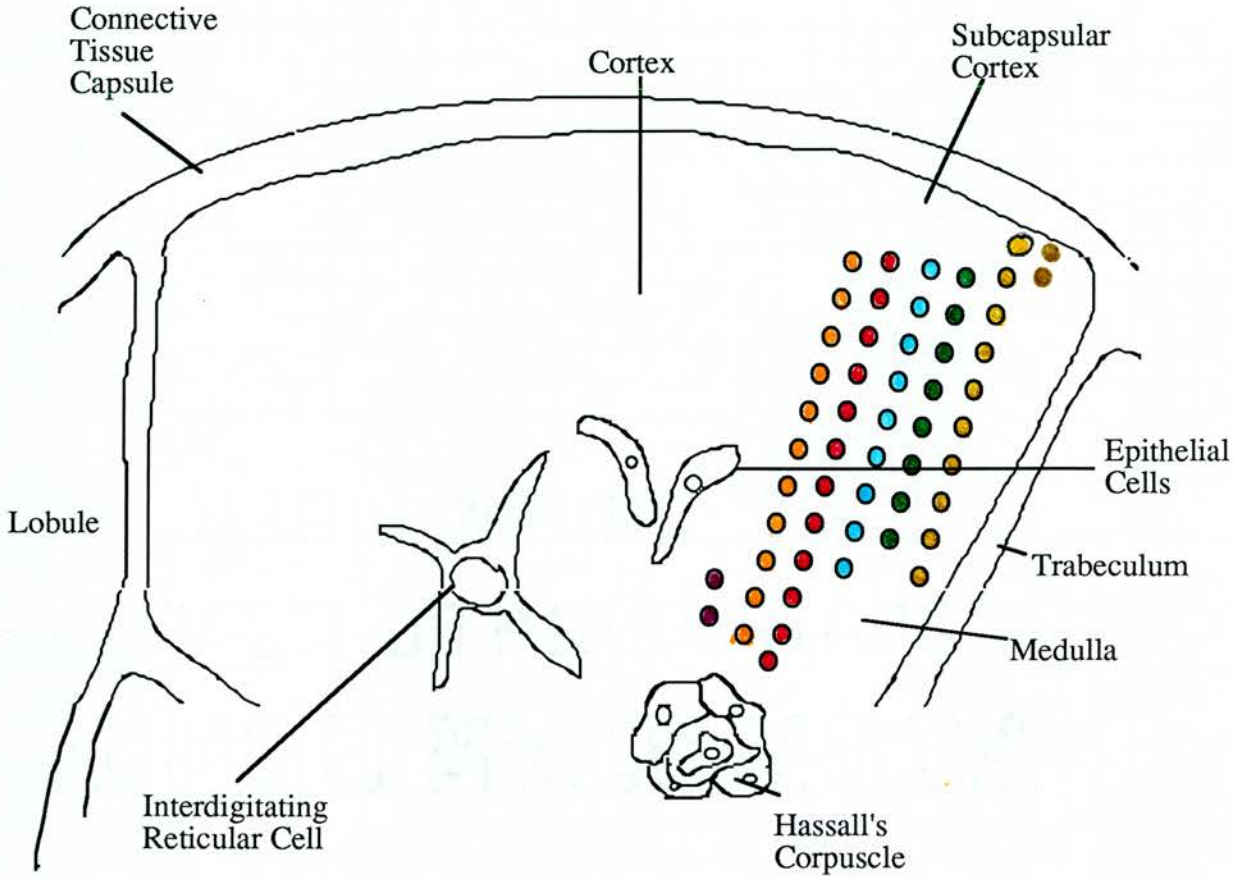

FIGURE 3: Human Thymus. Schematic Representation Of Distribution Of T Cell Differentiation Antigens

KEY: Expression of antigen by majority of cells expressed by circle.

- OKT9
- OKT10
- OKT6
- OKT8, Leu2A (TCS)
- OKT4, Leu3A (TH)
- Leu1 (PANT)
- OKT3 (PANT)

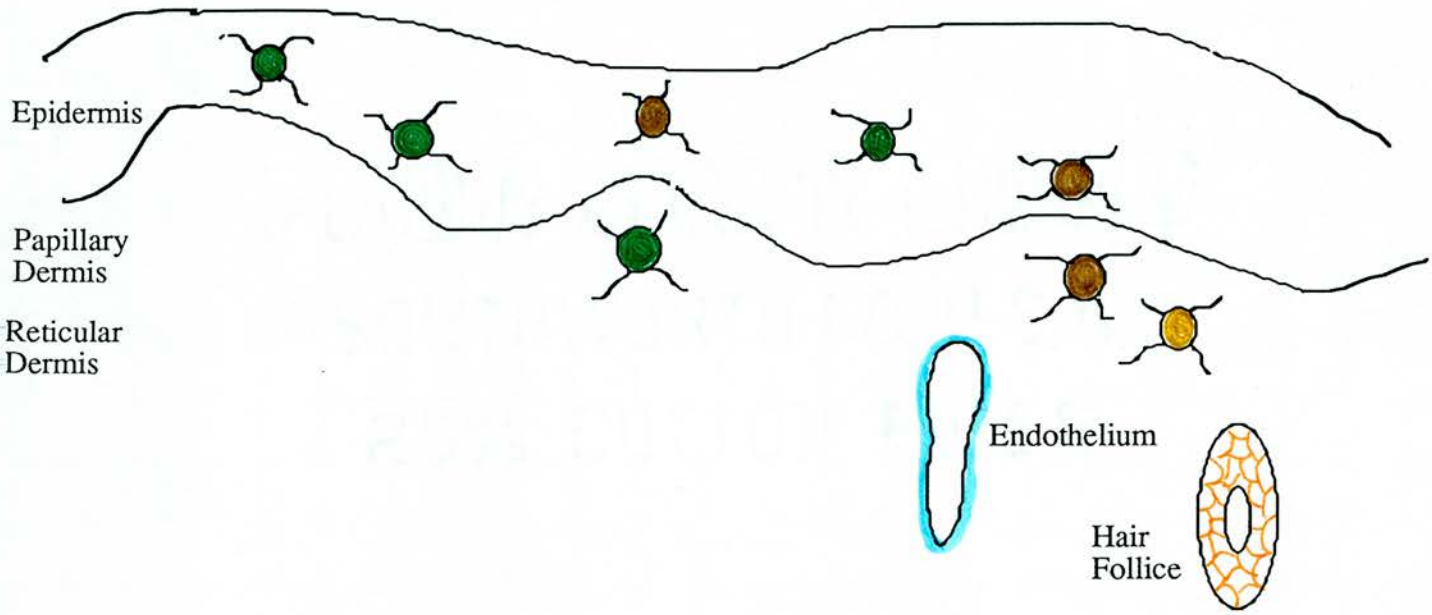

FIGURE 4. Reactivity of Monoclonal Antibodies on Normal Human Skin. Schematic Representation of Distribution of Monoclonal Determinants.

KEY.

OKT6 Common Thymocyte

HLADR

MPS (LeuM3, My3, My13)

Leukaemia Lymphoma Be2

Leukaemia Lymphoma Be1

Negative Reaction PANT (T11, OKT11A, T101, Leu1, OKT3)
T Subset (OKT4, OKT8, Leu3A, Leu2A)
K/NK (HNK1/Leu7)
Immature T (OKT9, OKT10)
Common Acute Lymphoblastic Leukaemia (J5)
Follicular Dendritic (R423)
Myeloid Leukaemia (MY10, MY12)
MPS (LeuM1)
Control (MOPC21)

NOTE. OKT6 and HLADR may be coexpressed on epidermal dendritic cells. OKT6, HLADR, and LeuM3, My3, My13 may be coexpressed on dermal dendritic cells

OKT10), common acute lymphoblastic leukaemia antigen (J5), follicular dendritic reticulum cell (R423), myeloid leukaemia (MY10, My12), monocyte-macrophage (LeuM1), and control MOPC21. Mouse ascites produces weak diffuse nonspecific staining of dermal collagen.

Positive reactions occur with the following: 1) Immature T cell (OKT6); epidermal dendritic cells and occasional dermal dendritic cells. 2) HLAD Locus (HLADR); epidermal dendritic cells but less frequently than OKT6; occasional dermal dendritic cells and endothelial cells. 3) Monocyte-macrophage (LeuM3, MY3, My13); weak staining dermal dendritic cells. 4) Leukaemia-lymphoma (Be1); follicular epithelium. 5) Leukaemia-lymphoma (Be2); dermal endothelium.

ESTERASE STAINING

Esterase positive macrophages are identified in LFs and IFAs of tonsil (Plate 18) and lymph node (including GCs and MZs of secondary LF). Only occasional weak staining cells are identified in CE of tonsil. Positive staining macrophages are present in cortex and medulla of thymus. Occasional positively staining macrophages are present in the reticular dermis of normal skin.

D. Discussion:

TONSIL AND LYMPH NODE:

The results obtained with mature T cell markers and B cell reagents indicate these populations have a distinct topographic localization and are consistent with current notions of T cell localization in IFAs and B cell localization in LFs (Gutman and Weissman, 1972). The finding of occasional T cells (OKT3+, Leu1+, T101+, OKT11A+, T11+) within LFs is interesting because E rosetting cells have previously been found in the neoplastic follicles of B cell lymphomas (Jaffe, et al., 1977), raising the possibility that the neoplastic follicle might mirror some of the cell/cell relationships of normal LFs. The retention

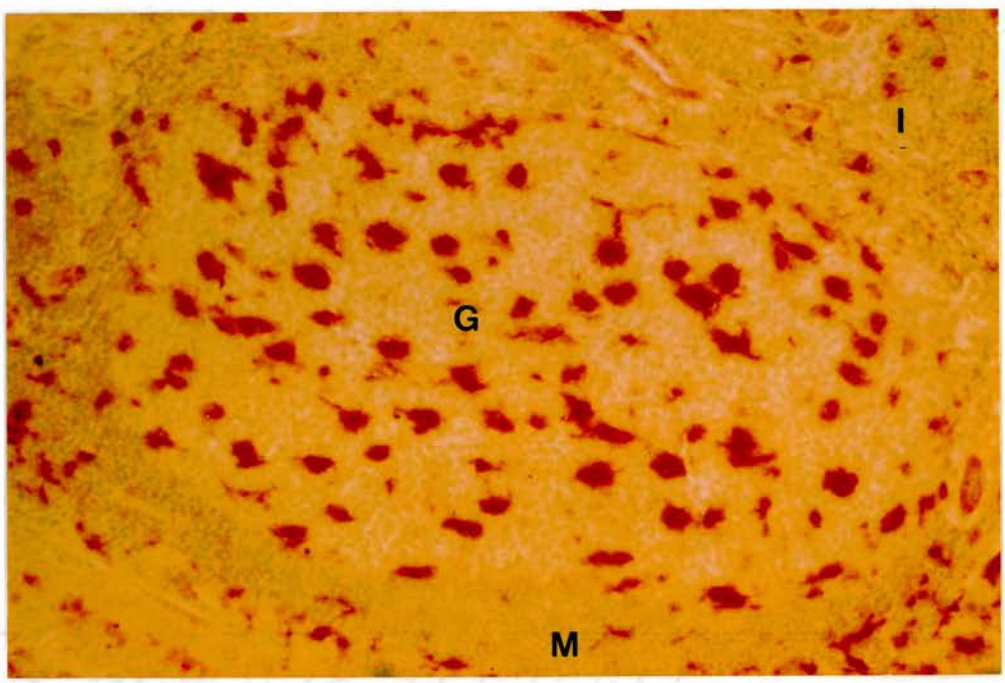

PLATE 18. **HUMAN TONSIL.** Esterase positive macrophages are numerous within germinal centre (G) of follicle with occasional positively staining cells within mantle zone (M) and interfollicular area (I). Alpha Naphthyl Acetate Esterase. Methyl green counterstain x 40.

of the capacity to form a nodular structure with some semblance of the physiologic follicles would presumably support this contention. T lymphocytes have previously been described in mouse lymph node germinal centres by Gutmann (Gutman and Weissman, 1972) and rarely in human tonsillar LFs by Lamelin when using heteroantisera and immunofluorescence (Lamelin, et al., 1978). The results here using a battery of highly specific reagents substantiate this phenomenon. The distribution of T subsets mainly within IFAs parallel the results obtained with Pan T cell reagents and indicate that helper cells are preponderant in tonsil and lymph node IFAs. The distribution of T subsets has not previously been reported. The presence of T subsets and peculiar distribution of OKT4/Leu3A (helper) cells in the crescentic pattern at GC/MZ junction may reflect areas of potential TB cooperation. Studies in mice (Rouse, 1982), reported at a later date, have confirmed the presence of cells with a "helper" phenotype in germinal centres. The crescentic pattern frequently noted with peripheral T cell and helper T cell antibodies is not evident with the OKT8/Leu2A (suppressor) antibodies. It is not known if this reflects a difference in the topographic distribution of helper and suppressor subsets or whether the absence of the crescentic pattern is merely due to the overall smaller number of suppressor cells present, making discernment of this pattern difficult.

It was mentioned earlier that lymphoma cells tend to mirror their normal counterparts in their metastatic migration patterns. For instance, CTCL, a helper T cell neoplasm (Berger, et al., 1979), causes expansion of the IFAs with spread of the disease (Long and Mihm, 1974). It will be interesting to see if any helper or suppressor neoplasia may occupy GCs.

Niewenhuis and Ford (Niewenhuis and Ford, 1976) demonstrated in radiolabeling experiments that circulating

rat B lymphocytes initially localize in IFAs before migrating to LFs. The presence of rare B1+ cells in human IFAs is consistent with their findings.

An interesting contrast is the finding of occasional intraepithelial cells staining positively with the T cell antibodies but not with the B cell monoclonals. The mechanism of this peculiar affinity of T cells for epithelium is unknown (Edelson, et al., 1974; McMillan, et al., 1981c), but is noted in later chapters when benign and malignant cutaneous T and B cell infiltrates are studied. In this context, it is of interest that numerous OKT6+ dendritic cells are present in CE. Prior to this study, the OKT6 antigen was thought to be confined to the thymus (Kung, et al., 1980; Reinherz and Schlossman, 1980) and acute T cell leukemic lymphoblasts (Reinherz, et al., 1980). The results here in tonsil, lymph node and in skin, indicate that OKT6 reactive cells may be found in extrathymic locations in non-malignant situations. OKT6 reactivity appears to be a property of Langerhans' Cells (McMillan, et al., 1981b; Fithian, et al., 1981; Murphy, et al., 1981) which have an important role in presenting antigen to T cells (Silberberg-Sinakin, et al., 1978; Hunter, 1983). The proximity of OKT6+ dendritic cells to exocytic T Cells (OKT3+, Leu1+, T11+, OKT11A+, T101+) in crypt epithelium is compatible with this role.

Cells with a suppressor phenotype (Leu2A+/OKT8+) are more easily identified in CE than Leu3A+ helper cells. It is unknown whether this reflects a difference in the density of the appropriate antigens or a true difference in the affinity of these subsets for epithelium. If the latter is the case, then this indicates an interesting contrast between reactive and neoplastic states because helper T cell lymphomas are notoriously epidermotrophic (Chapter 5). A similar epitheliotropism of cells with suppressor phenotype (Lyt1-23+) has been noted in delayed hypersensitivity

reactions in mice (Tigelaar, 1983) and skin allografts undergoing rejection in humans (Bhan, et al., 1982).

The prevalence of Leu3A+/OKT4+ over Leu2A+/OKT8+ cells in the IFAs of human lymph nodes and tonsils makes it unlikely that helper-suppressor (H/S) ratios will be useful in detecting early nodal involvement in helper T cell neoplasia; this has been borne out by two preliminary studies (Willemze, et al., 1985; Burke, et al., 1986). The subset pattern in lymph node and tonsil contrasts with spleen where Leu3A+ cells are predominantly in the periarteriolar lymphocyte sheath and Leu2A+ cells are almost restricted to the cords of Billroth in the red pulp (VanDerValk, et al., 1984a).

The nature of the OKT6+ cells in two of five lymph nodes requires comment. The dendritic pattern suggests a histiocytic subpopulation. Three histiocytic cell types might account for OKT6 reactivity: a) a macrophage analogous to Nossal's follicular dendritic reticulum cell (Nossal, et al., 1968) b) the Langerhans' cell (McMillan, et al., 1981b; Fithian, et al., 1981; Murphy, et al., 1981) and c) the interdigitating reticulum cell (Veldmann, 1970; Veerman, 1974; Rausch, et al., 1977).

a) Nossal's follicular dendritic reticulum cells are located in the corona of lymphoid follicles of mice. As no staining was observed in primary or secondary LFs, OKT6 was not apparently staining a subpopulation of histiocytes analogous to Nossal's cells (CF results with R423).

b) OKT6 reactivity with cells of Langerhans' lineage (i.e. Langerhans' cells or indeterminate cells) has been mentioned already, and a recent study by Weiss et al. (Weiss, et al., 1986) has demonstrated the Leu6+ nodal population to be HLADR and S100 positive (Leu6 has a reactivity similar to OKT6). Langerhans' cells have been occasionally described in normal human lymph node (Vernon, et al., 1973; Rausch, et al., 1977) and especially in lymph node undergoing antigenic

challenge eg., dermatopathic lymphadenopathy (Jimbow, et al., 1969). It is not known if the two patients with OKT6+ nodal cells had cutaneous disease. The lymph nodes were, however, reported as "normal" in surgical pathology and showed no evidence of dermatopathic lymphadenopathy when re-examined. The number of cells staining positively with OKT6 in lymph nodes is small, and, therefore, compatible with their being Langerhans' cells.

The dendritic reaction obtained in tonsil with OKT6 also suggests that the positively reacting cells are Langerhans' cells since Langerhans' cells have previously been described in oral epithelium (Waterhouse and Squier, 1967) and tonsil (Wood, et al., 1985).

c) The third histiocytic candidate accounting for OKT6 reactivity is the interdigitating reticulum cell which is found in the IFA of LNs (Rausch, et al., 1977) and may also be involved in interactions with T lymphocytes (Veerman, 1974); Rausch, et al., 1977). This cell is closely related morphologically to the Langerhans' cell (Rausch, et al., 1977) and may only be differentiated ultrastructurally from Langerhans' cells by its absence of Birbeck granules (Rausch, et al., 1977). OKT6 dendritic reactivity was only found in the IFAs of two of five lymph nodes and in none of the tonsils examined. OKT6 reactivity would, therefore, be far from a universal marker of interdigitating reticulum cells (DRCs) in contrast to S100 which stains a large number of dendritic cells in the T cell zones of lymph nodes (Wood, et al., 1985). The acquisition of OKT6 reactivity in T accessory dendritic cells therefore appears to be a function of their epithelial micro-environment (Murphy, et al., 1986).

The distribution of HNK1+ cells in non-neoplastic lymphoid tissue has still to be fully characterized. HNK1+ lymphocytes are readily observed in LFs and these results suggest that tonsil will be a useful positive control for

phenotyping of neoplasia, even though the overall number of HNK1+ cells in human tonsil is small. The presence of HNK1+ lymphocytes in tonsil will permit its use as a positive control in initial attempts at studying the prevalence of these cells in human cancer. Recently, a neoplasm of killer cells has been described (Ferrarini, et al., 1983). It remains to be seen whether killer-cell neoplasia would initially metastasize to or preferentially expand lymphoid follicles. It has also recently been shown that HNK1+ lymphocytes may be OKT3+ during their ontogeny (Abo and Balch, 1981), and it is already known that killer/natural killer (K/NK) cells may show weak expression of the E receptor (Herberman and Ortaldo, 1981). It is, therefore, possible that some of the OKT3+, T11+, OKT11A+ cells in lymphoid follicles might be K/NK lymphocytes, rather than conventional T cells.

The presence of HLADR antigen on a wide range of cells (B cells, monocytes, macrophages, Langerhans' cells, and activated T cells) (Warnke and Levy, 1980) probably explains the distribution observed here with positive staining in LFs, IFAs, and CE.

The positive reaction (mainly in LFs) with OKT9 is of particular interest. This antigen was originally produced by immunizing mice with T-cell acute lymphocytic leukaemia (ALL) cells (Kung, et al., 1980) and is present in a subcapsular thymic population (see results in Thymus) and mitogen-activated T cells (Kung, et al., 1980). The presence of OKT9 on a wide variety of replicating cell types, including T cell ALL (Kung, et al., 1980) and cutaneous T-cell lymphoma (Chapter 5) suggests that monoclonal OKT9 may be useful in immunotherapy. The OKT9 antibody labels the transferrin receptor (Greaves, et al., 1981). The results here, however, suggest that this antigen is probably expressed by a large number of cells in normal reactive tissue and suggest significant side effects if OKT9

is used in this manner. The large number of LFs staining here with OKT9 indicates the probable expression of OKT9 on cells of B lineage and some macrophages. Reaction of OKT9 with tingible body macrophages has also been reported by Hsu et al (Hsu and Jaffe, 1984a).

The monoclonal determinant OKT10 has been found in immature thymocytes (Kung, et al., 1980), (see results in Thymus) bone marrow progenitor cells, some activated T cells, and T-cell ALL. (Kung, et al., 1980) The exact nature of the OKT10+ cells observed here in tonsil remains to be seen. A similar mixture of membranous and cytoplasmic staining in lymphoid follicles has been noted by other groups (Bhan, et al., 1981a; Hsu and Jaffe, 1984a). The results, therefore, confirm that this antigen has a broader distribution than confinement to immature thymocytes and T-cell ALL. The same provisos concerning McAb therapy with O<T10, therefore, stand as with OKT9.

Occasional cells in LFs react positively with the monocyte antibodies (OKM1, My3, My13, MO2, LeuM3). The staining tends to be variable, however. Similar distributions in tonsil for OKM1 (VanDerValk, et al., 1984a; Knowles, et al., 1984) and LeuM3 (Hofman, et al., 1984; Wood, et al., 1985) have recently been noted. Whether tonsils will prove to be the most satisfactory control tissue in phenotyping lymphomas for monocyte or myeloid differentiation antigens remains to be seen. LeuM1+ cells are uncommon and tend to be found at random throughout IFAs. A contrasting distribution of LeuM1+ cells (IFAs) versus OKM1+ cells (LFs, IFAs, medullary sinuses) has also been recorded by Hofman, et al. (Hofman, et al., 1984) The findings with the monocyte-macrophage antibodies, therefore, suggest the presence of monocyte-macrophage subpopulations occupying different microenvironments in vivo (CF distribution of OKM1, LeuM3, MO2, and LeuM1 in Table 3). Alternatively, these antigens may be restricted to varying

stages of monocyte maturation in a manner analogous to the patterns of immunoglobulin expression seen on B cells during their stages of differentiation.

As expected, the R423 monoclonal produces positive staining of dendritic cells in LFs but not in IFAs or CE. The distribution of R423+ cells, therefore, contrasts sharply with the OKT6+ dendritic population, which is confined to CE. These results are consistent with previous notions of different subpopulations of dendritic ("D-cell") subpopulations in normal lymphoid tissue (Tew, et al., 1982). The dendritic reticulum (follicular dendritic) cell is thought to be involved in antigenic capture for B cells (Mandel, et al., 1980). The OKT6 antigen is found on Langerhans' cells, which are involved in antigenic processing of T-cell-mediated immune reactions (Silberberg, et al., 1978).

An equivocal or negative reaction is obtained with J5 in all specimens. This antibody detects the common acute lymphoblastic Leukaemia antigen (CALLA) (Ritz, et al., 1980), which is present on non-T, non-B (null) cell leukaemias. The distribution of the J5 antigen, therefore, contrasts with the other "immature" markers OKT9 and OKT10, which, although present in some leukaemias, have also been shown here to be readily demonstrable in tonsil. J5 reactivity may be more prevalent in lymphoid tissue than these results suggest, however, because Hsu, et al., (Hsu and Jaffe, 1984a) using a more sensitive technique, recorded hazy diffuse staining in germinal centre cells and some cells in MZs and T cell zones.

The My antibodies produce contrasting results. My10 and My12 produce a negative reaction, which suggests that the antigens detected by these reagents are not prevalent in nonneoplastic lymphoid tissue. Further investigation of the distribution of these determinants in normal tissues would be worthwhile, because they may be relatively specific for

malignant cells. My11 stains a large number of cells in LFs and IFAs. This is consistent with the presence of the respective antigen on peripheral blood lymphocytes (PBLs) positive and negative for E rosettes. My3 and 13 react with intrafollicular macrophages.

The leukaemia-lymphoma antibodies Be1, Be2 produce negative staining in lymph nodes but in tonsil Be2 produces positive staining in the interfollicular area (Be1 reaction negative). Tonsil was not included in the original screening studies of these antibodies. (Berger, Morrison, Chu, et al., 1982) The results indicate that reactive lymphoid tissue may express the Be2 antigen in a small proportion of cells. The Epstein Barr virus status of the donors of the tonsils concerned is unknown.

MOPC21 does not have any known specificity and produced a negative reaction in the tonsils examined. This antibody will probably be very useful as a negative control in immunophenotyping of the lymphoreticular system.

THYMUS (TABLE 4, FIGURE 3)

The results in thymus are consistent with Reinherz's scheme of intrathymic differentiation (Table 5) with gradual acquisition of some antigens and loss of others as thymocytes mature and migrate from deep cortex to medulla. The OKT9, OKT10, and OKT6 antigens are readily demonstrated in thymic cortex (Plates 19 and 20) but not in thymic medulla where the cell phenotype OKT3+, Leu1+, Leu3A+/Leu2A+, OKT6-, OKT9-, OKT10- is similar to that of resting mature peripheral T cells found in peripheral blood (Kung, et al., 1980) and the T cell zones of peripheral lymphoid tissue (Table 4).

The finding of Leu3A/OKT4 and Leu2A/OKT8 staining of the majority of cortical thymocytes implies the presence of cortical thymocytes simultaneously expressing "helper" and "suppressor" antigens. This situation again contrasts with

TABLE 5

Scheme of Intrathymic Differentiation in Humans

Adapted from Reinherz et al. (Reinherz, Schlossman, 1980)

Stage I	Thy1	OKT10+
Early	Thy2	OKT10+
Thymocyte		OKT9+
	via Thy 3	
Stage II	Thy 4	OKT10+
Common		OKT6+
Thymocyte		OKT4+
		OKT5+
		OKT8+
	via	
	Thy 5 and Thy 6	
Stage III	Thy 7	Thy 8
Mature		
Thymocyte	OKT10+	OKT10+
	OKT1+	OKT1+
	OKT3+	OKT3+
	OKT4+	OKT5+
		OKT8+
Stage IV		
Mature	OKT1+	OKT1+
Peripheral	OKT3+	OKT3+
T Cell	OKT4+	OKT5+
		OKT8+
	(HELPER)	(CYTOTOXIC/SUPPRESSOR)

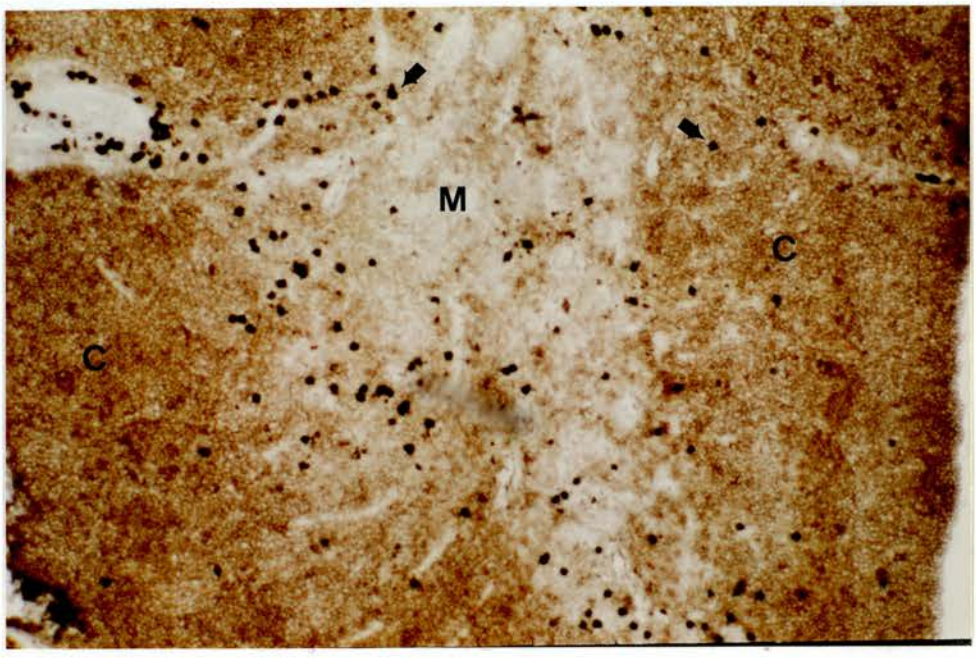

PLATE 19. **HUMAN THYMUS.** Common thymocyte antibody OKT6 stains majority of thymocytes in thymic cortex (C) whereas medulla (M) shows weak staining. Thymic macrophages are evident by their endogenous peroxidase activity (arrow). Indirect immunoperoxidase. Non counterstained x 25.

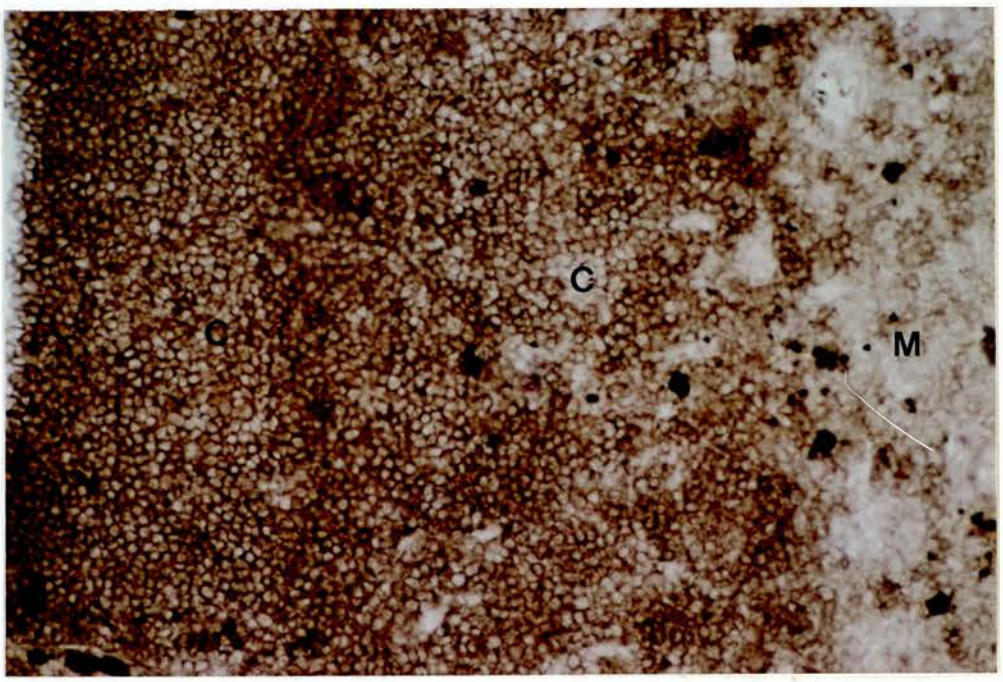

PLATE 20. **HUMAN THYMUS.** High power. Thymic cortex (C) stains positively with OKT6 whereas medulla (M) only shows occasionally weakly staining cells. Indirect immunoperoxidase. Non counterstained x 64.

circulating peripheral blood T cells which are generally either Leu3A+/OKT4+ (helper) or Leu2A+/OKT8+ (suppressor).

The significance of cells bearing these immature or double markers in malignant T cell lymphoma will be discussed later (Chapter 5).

The presence of rare dendritic forms expressing the OKT6 marker in thymic cortex is worth further investigation. Cells containing Birbeck granules have been previously noted in ultrastructural studies of human thymus (Hoshino, et al., 1970). Consideration of the antigen handling function of Langerhans' cells for T cells in cell mediated immune reactions (Silberberg, et al., 1975) makes it tempting to speculate that Langerhans' cells may be involved in some type of information transfer integral to intrathymic T cell development. The presence of Langerhans' cells within the thymus and the presence of the OKT6 determinant on Langerhans' cells and immature T cells also suggests the possibility of a more direct developmental link between Langerhans' cells and T cells. This notion is not inconsistent with evidence that epidermal Langerhans' cells are derived from bone marrow derived monocytes (Katz, et al., 1979). Presumably a common bone marrow derived stem cell might migrate to the thymus and skin and differentiate into OKT6+ Birbeck granule-(indeterminate)/Birbeck granule+ (Langerhans') cells. The latter, under the inductive influence of the thymus might influence the development of or actually differentiate into T cells.

In contrast to the rare OKT6+ dendritic cells demonstrated here an Ia+ epithelial reticular network is relatively easily demonstrated in human thymus (Janossy, et al., 1980) and there is evidence for a role of these dendritic cells and macrophages in T-cell development (Wekerle, et al., 1973; Beller and Unanue, 1978).

Concurrent studies of tonsil (Bhan, et al., 1981a; Hofman, et al., 1983a; Si, et al., 1983; Feller, et al.,

1983; Hsu and Jaffe, 1984a and b; Wood, et al., 1985), lymph node (Poppema, et al., 1981), and thymus (Bhan, et al., 1980; Hsu and Jaffe, 1985) in other laboratories have produced similar findings to those outlined here.

A paper has recently appeared (VanDenOord, et al., 1985) indicating a similar paucity of reactivity of interdigitating reticulum cells with OKT6 but demonstrating an increase of OKT6+ cells in dermatopathic lymphadenitis. The distribution of cell subsets in human spleen (Stuart and Warford, 1983; Timens and Poppema, 1985) has also been reported. The main differences from the pattern in lymph node and tonsil were those already described for Leu3A/Leu2A (page 80) and the reactivity of OKT8 with splenic sinusoids. (Stuart and Warford, 1983)

SKIN

The negative reaction with B cell and mature T cell/subset McAbs is expected and confirms the specificity of these reagents for lymphoid cells, which tend to be infrequent passengers through normal skin.

The negative staining with immaturity antigens OKT9, OKT10 contrasts with tonsil where OKT9, OKT10 positivity is recorded on lymphocytes (OKT9, OKT10) and macrophages (OKT9). This indicates the utility of screening new McAbs with multiple organs. The common thymocyte antibody OKT6 produces surprising cross reactivity with epidermal dendritic cells. Prior to these studies, and those in Chapter 5, OKT6 was thought to be specific for common thymocytes (Kung, et al., 1980). These studies were among the first to demonstrate such crossreactivity. Simultaneous studies in independent laboratories have shown the OKT6 positive epidermal dendritic cells to be Ia (HLADR) positive (Fithian, et al., 1981) and to contain Birbeck granules (Murphy et al., 1981) indicating their Langerhans' cell nature.

The reactivity of epidermal and dermal dendritic and

endothelial cells with HLADR confirms previous findings. (Rowden, 1977) Epidermal HLADR+ cells are less frequent than OKT6+ cells and this has been noted by other workers (Harrist, et al., 1983a).

The positive reaction of dermal macrophages with My3, My13, LeuM3 (negative epidermal dendritic reaction) and preponderant localization of OKT6 reactivity to epithelium is reminiscent of tonsil and again suggests the existence of subsets of MPS cells or preferential expression of certain antigens on MPS cells during their lifespan.

The negative reaction with R423 in contrast to the above MPS McAbs is consistent with confinement of this marker to follicular dendritic cells (FDC). Results in Chapters 6 and 7 indicate, however, that R423+ FDC may be found in the skin in certain pathological states where B cell migration is disturbed.

The negative reaction with leukaemia associated antigens J5, My10, My12 along with similar results in tonsil suggest a limited distribution of these antigens in non-malignant tissue and that further testing would be useful to characterize their specificities and any cross reactivities with normal tissue components.

The reactivity of Be1 with follicular epithelium and Be2 with endothelial cells again broadens the spectrum of reactivity of these McAbs beyond lymphomas and malignant cell lines. As demonstrated in Chapters 5 and 7 these two antigens may also be expressed on reactive lymphoid cells.

II. POSITIVE PATCH TESTS IN ALLERGIC CONTACT DERMATITIS

A. INTRODUCTION

The requirement for comparison between reactive and malignant states; the theory that mycosis fungoides (one type of cutaneous lymphoma) is due to a persistent antigenic

stimulus; and the morphologic similarities between "mycosis" cells and transformed lymphocytes suggests that a study of contact dermatitis would be worthwhile. Additional information concerning the immunopathology of contact dermatitis might also be obtained.

The characteristics of immune infiltrating cells in allergic contact dermatitis are studied using McAbs directed against mature T cells, T cell subsets, immature differentiation antigens, Killer (K) and Natural Killer (NK) cells, B cells, Langerhans' cells and common acute lymphoblastic leukemia antigen (CALLA).

B. MATERIALS AND METHODS

Thirteen positive patch tests from 8 patients with allergic contact dermatitis and 1 lesional biopsy of poison ivy were studied. The "positively reacting" substances were rosin, paraphenylene diamine (PPD) mix, isopropyl-n-phenyl paraphenylene diamine (IPPD), fragrance, furasizone, mercapto mix, (N-Cyclohexy 1-2-benzothiazole sulfenamide 2,2-Benzothiazyl Disulfide, 4-morpholinyl-2-Benzothiazyl Disulfide), ammoniated mercury, merthiolate (2 cases), chromate, nickel (2 cases), and neomycin. Patch tests were read at 48 hours, and 3 mm punch biopsies taken of positive patches. The McAbs used include Leu1, T11, Leu2A, Leu3A, B1, HLADR, OKT6, OKT9, OKT10, HNK1, J5, R423, Be1 and Be2; their source and specificity is outlined in Chapter 3 Table 2.

C. RESULTS (Figure 5)

Light microscopy of haematoxylin eosin stained sections show features of allergic contact dermatitis with epidermal spongiotic oedema, exocytosis (mainly lymphocytic), and microvesicle formation. Epidermal changes are focal and spotty horizontally and vertically. The dermis exhibits variable oedema with a papillary, subpapillary and perivascular lymphohistiocytic infiltrate.

In cases examined, >50% of the infiltrating cells in

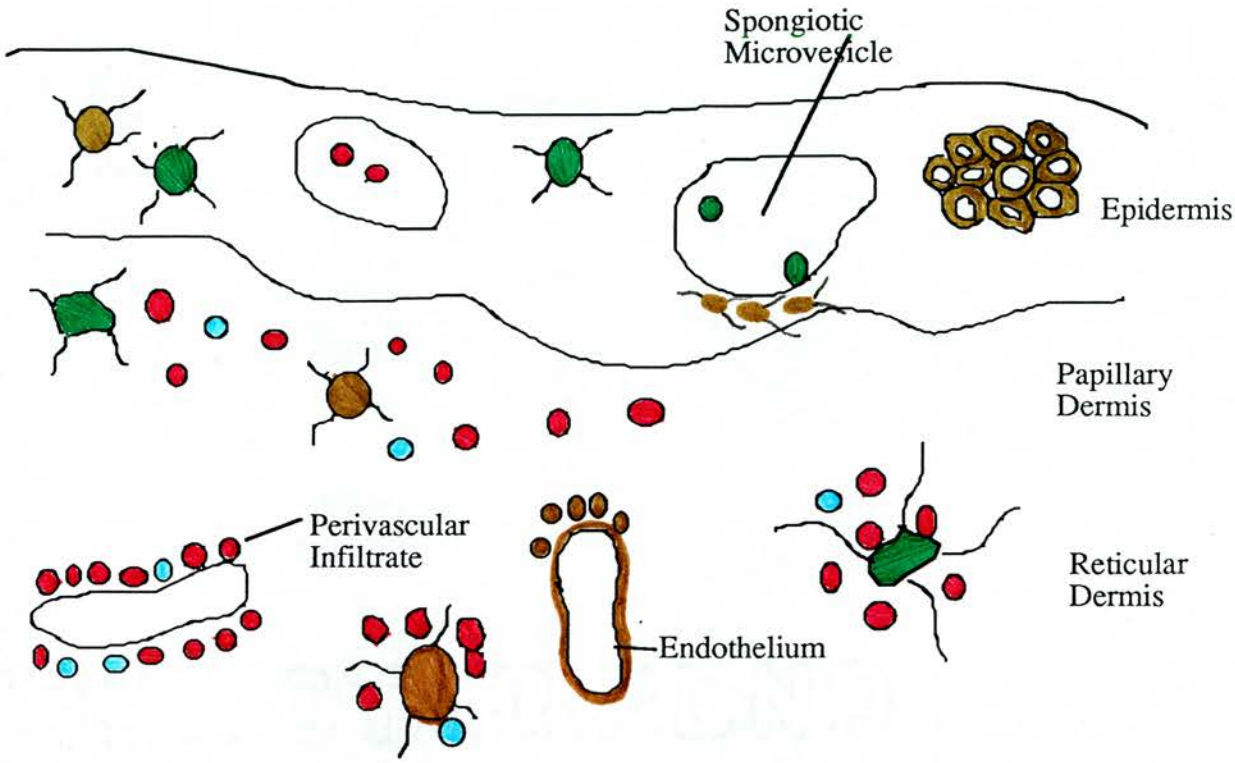

FIGURE 5. **Schematic Representation Of Phenotype Of Immune Cells In Allergic Contact Dermatitis.**

KEY

- OKT 6
- HLADR
- T Helper (Leu3A)
- T Cytotoxic/Suppressor (Leu2A)
- \+ PANT (Leu1, T11)
- HLADR Positive Keratinocyte

Note that certain markers may be coexpressed e.g. HLADR, OKT6 on dendritic cells, HLADR and PAN T or T subset markers on T cells. These are shown separately in schematic diagram in a manner analogous to single labelling studies from which information was derived.

dermis and epidermis react with Leu1 (Pan T) and T11 (E receptor). The majority of these cells are Leu3A+ (helper), the helper/suppressor ratio varying from 2:1 to 10:1, larger numbers of helper cells being observed in denser infiltrates. The HLADR antibody produces patchy positive membranous staining of epidermal keratinocytes. A more intense level of positive staining with HLADR is also noted in (1) epidermal dendritic cells: (2) cells surrounding and within spongiotic microvesicles (Plate 21, 22); these consist of rounded and dendritic forms; (3) dermal cells; isolated HLADR+ dendritic cells are observed throughout the dermis (Plate 23) (especially in the papillary and superficial reticular dermis). HLADR+ dendritic cells are also interspersed throughout the perivascular mononuclear cell infiltrates. HLADR positivity with a confluent pattern is also observed throughout the perivascular mononuclear cell infiltrates indicating that the majority of the Leu1+ and T11+ cells are also HLADR+ (although the density of antigenic staining is less than in the HLADR+ dendritic cells). The confluent HLADR positivity of perivascular cells tends to vary in intensity both within the same specimen and among different biopsy specimens.

The OKT6 antibody reacts with epidermal dendritic cells, cells within microvesicles (Plate 24), and dendritic cells lying free and interspersed throughout the dermal mononuclear infliltrates. The OKT6+ dendritic cells account for 5-10% of the infiltrating dermal cells.

Only occasional cells (<1%) react with McAB B1 (B cell).

HNK1+ lymphocytes are only seen in 1 case, forming less than 1% of the cell population and occurring in the dermis and epidermis (including spongiotic microvesicles).

A negative reaction is obtained with OKT9 (immature) OKT10 (immature) J5 (CALLA) and R423 (Follicular dendritic cell antibodies. Be1 produces a positive reaction in 1 of 4

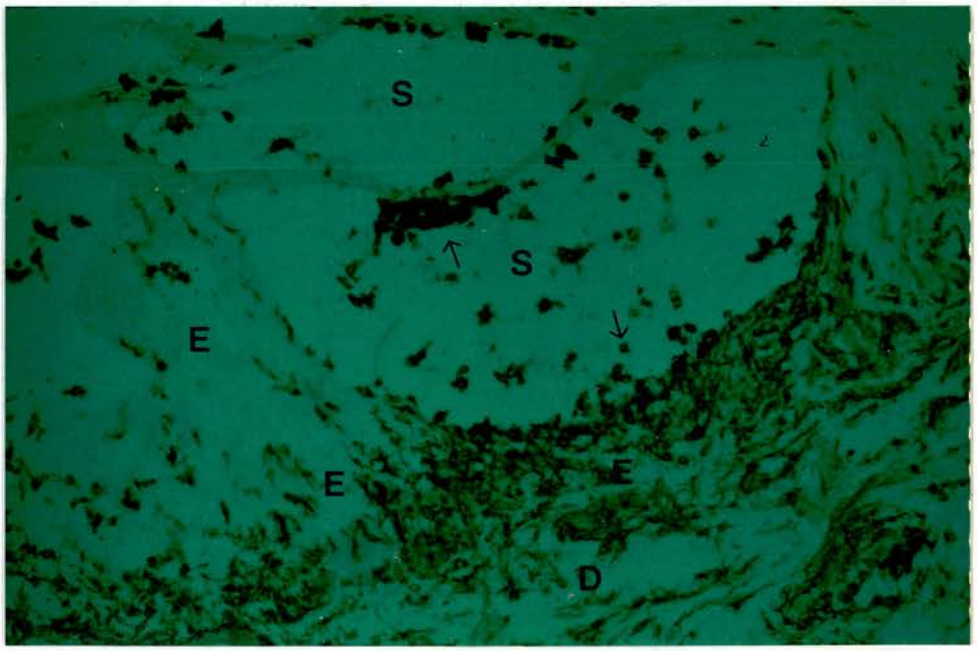

PLATE 21. **ALLERGIC CONTACT DERMATITIS.** HLADR+ cells around and within spongiotic microvesicles (S) HLADR+ cells in upper dermis (D) and lower epidermis (E) subjacent to lowest vesicle mainly show dendritic morphology whereas HLADR+ cells within vesicle (arrow) display more rounded morphology. Indirect immunoperoxidase. Non counterstained x 40.

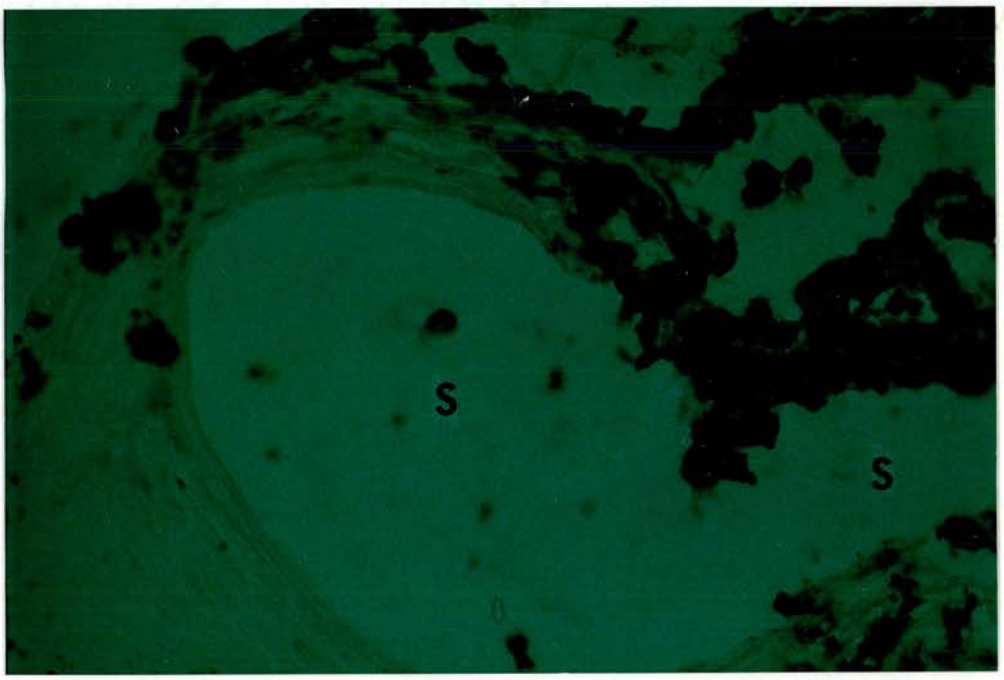

PLATE 22. **ALLERGIC CONTACT DERMATITIS.** Densely staining mainly rounded HLADR+ cells adjacent to and within spongiotic microvesicle (S). Indirect immunoperoxidase. Non counterstained x 100.

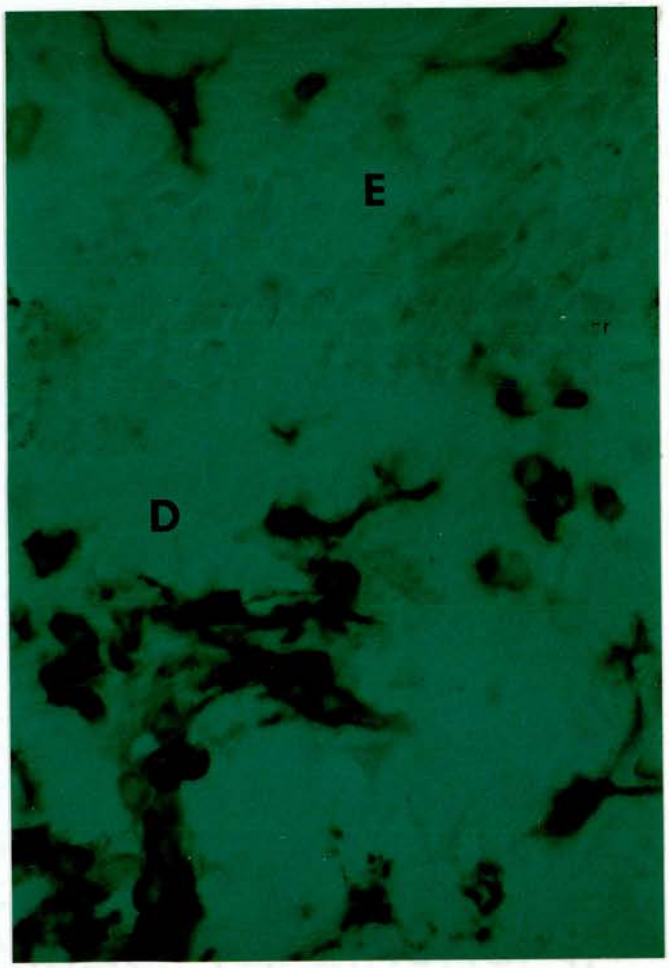

PLATE 23. **ALLERGIC CONTACT DERMATITIS.** High power of dendritic staining HLADR+ cells in dermis (D) and epidermis (E). Indirect immunoperoxidase. Non counterstained. Green filter x 160.

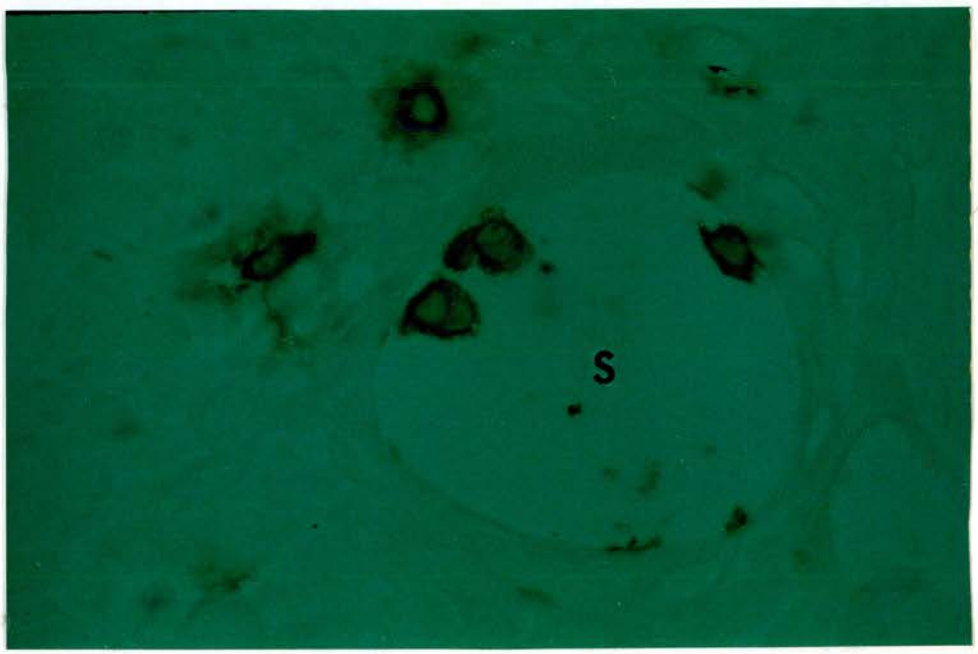

PLATE 24. **ALLERGIC CONTACT DERMATITIS.** Epidermal OKT6+ cells on inner edge and adjacent to spongiotic microvesicle (S). Indirect immunoperoxidase. Non counterstained. Green filter x 160.

positive patch (Nickel) tests (10% of infiltrating lymphoid cells). Be2 reaction is negative in 3 of 3 cases tested.

Positive controls (tonsil, cutaneous lymphoma, including one J5 positive specimen) are positive. All negative controls (Mouse ascites as primary antibody, omission of primary antibody, substitution of goat antirabbit Ig G for goat antimouse Ig G, and DAB alone) are negative. The use of multiple primary antibodies of different known specificities also serves as an internal control.

D. DISCUSSION

This study shows the majority of infiltrating lymphocytes in allergic contact dermatitis to have a T11+, Leu1+, Leu3A+ phenotype. As far as mature T cell markers are concerned, the phenotype is similar to that of cutaneous T cell lymphoma (CTCL) and many cases of benign dermatoses (Chapter 5). The results suggest that the effector cells in allergic contact dermatitis have the phenotype of mature T helper cells. This is compatible with one study of cell-mediated immunity (PPD intradermal skin testing) where the infiltrating cells have been shown to have an OKT3+, OKT4+ helper phenotype (Klareskog, et al., 1982) and other studies of contact dermatitis (Haynes, et al., 1982a; Scheynius, et al., 1983; Ralfkiaer and Wantzin, 1984). The infiltrating cells in various forms of inflammatory myopathy have also been shown to have a similar Leu1+, Leu3A+ phenotype (Feeback, 1982). The demonstration of a Leu1+, Leu3A+ phenotype in the infiltrating cells of irritant contact reactions (Scheynius, et al., 1983; Gawkrodger, et al., 1986) suggests that Leu3A positivity probably cannot be used to differentiate the allergic from the irritant model.

The staining pattern obtained with HLADR antibody in epidermis is compatible with the presence of HLADR antigen on Langerhans' cells and epidermal cells (Rowden, 1977). Similar findings have been reported by Gawkrodger, et al. (1986). Major histocompatibility complex (MHC) antigens are

divided into two groups, class I and class II. Class I antigens, human lymphocyte antigens (HLA)-A, B, and C are expressed on all adult nucleated cells. Class II antigens, the HLA-D series are mainly expressed on immune-competent cells; eg., B cells, activated T cells, macrophages, Langerhans' cells, endothelial cells. Normal epidermal class II positive cells include Langerhans' cells (Rowden, et al., 1977; Klareskog, et al., 1977) and occasional keratinocytes especially those of acrosyringeal epithelium (Murphy, et al., 1983; Carr, et al., 1986). The functional significance of keratinocyte class II expression is unknown. Several authours have suggested that HLADR expression may allow keratinocytes to act as antigen-presenting cells (Lampert, et al., 1981; Suitters, et al., 1982; Tjernlund and Scheynius, 1986). The HLADR expression is probably mediated by gamma-interferon (Scheynius, et al., 1986) released from immune competent cells (Lampert, 1981; Nickoloff, et al., 1985).

It is uncertain how many of the rounded HLADR+ cells within spongiotic microvesicles are Langerhans' cells or activated T cells. The confluent staining pattern produced by HLADR on the infiltrating dermal T cell population suggests that many of the cells have the phenotype of activated Tcells (Evans, et al., 1978). Some of the dermal HLADR reactivity is undoubtedly contributed by Langerhans' cells. The preponderantly negative reactions with monoclonal antibody B1 indicates that HLADR positivity is not mainly due to B cells.

OKT6+ dendritic cells are noted throughout the epidermis including spongiotic microvesicles. They are also noted apparently lying free within the dermis and throughout the dermal mononuclear infiltrates (which consist preponderantly of mature Leu1+, T11+ T cells) in a manner similar to other T cell infiltrates. The pattern of OKT6 reactivity indicates that HLADR reactivity observed is not

due solely to positively staining Langerhans' cells, substantiating that HLADR reactivity also resides on the T cell population. Although studies of this kind do not give direct information on cell function, the close approximation of OKT6+ cells to T cells provides evidence that Langerhans' cells may play an important role in contact dermatitis in humans. There is certainly good animal evidence that these cells function in the handling and presentation of antigen to T cells (Silberberg-Sinakin, et al., 1978), and lymphocytes have been noted in close apposition to Langerhans' cells in contact dermatitis in animals (Poulter, 1983). The role of antigen presenting cells in delayed-type hypersensitivity has recently been reviewed (Poulter, 1983). The OKT6+ dermal cells may account in part for the OKT3- histiocytes found adjacent to lymphocytes in contact allergic reactions (Panfilis, et al., 1983).

The positive reaction with OKT6 contrasts with the negative reaction with another dendritic cell marker R423. The reaction obtained with monoclonal antibodies B1 and R423 is consistent with current concepts of the relative importance of B cells (and their accessory cells) in humoral immunity and T cells in cell-mediated immunity (Weissman, et al., 1978). However, biopsies of patch tests taken at a later stage in allergic contact reactions may show a significant B cell component with the formation of germinal centres containing follicular dendritic cells (Ralfkiaer and Wantzin, 1984). The explanation for this may lie in the role of B cells in immunologic memory (Ralfkiaer and Wantizin, 1984).

Only rare cells in epidermis and dermis are noted to react with the monoclonal antibody HNK1 and only in one case. HNK1+ reactive cells are apparently uncommon in ACD since one other study (Ralfkiaer and Wantzin, 1984) failed to demonstrate them in any cases examined. K and NK cells are believed to have an important function in immunity of

animals against tumours and viruses. (Herberman and Ortaldo, 1981) Their importance in humans is less clear (Herberman and Ortaldo, 1981). Although several types of cells are involved in cellular cytotoxicity (Herberman and Ortaldo, 1981), this study indicates that the damage in contact dermatitis is probably mediated via classical T lymphocytes rather than K or NK cells, which apparently have properties of both monocytes (OKM1+) and T cells (weak E rosette positivity)(Herberman and Ortaldo, 1981).

These results are also compatible with animal evidence that contact sensitivity and cellular cytotoxicity are mediated by different lymphocyte subclasses (Dennert and Hatlen, 1975; Huber, et al., 1976) as only occasional cells produce a positive reaction with the cytotoxic suppressor cell antibody Leu2A. It must be emphasized that reactions were studied only at 48 hours and that sequential testing may reveal variation in the distribution and quantity of various subpopulations (Gawkrodger, et al., 1986). For instance cells with a suppressor phenotype may be more common in later stages of developing or resolving (spontaneous or iatrogenic) lesions.

The negative reaction obtained with OKT9 and OKT10 is of great interest. Haynes, et al. (Haynes, et al., 1982a) also found a negative reaction for the transferrin receptor in allergic contact dermatitis when using a different McAb, 5E9. Both these antigens may be expressed by a proportion of the infiltrating cells in CTCL (McMillan, 1985) Chapter 5). This may be due to immaturity or "dedifferentiation" from neoplastic transformation (Chapter 5). Since both the OKT9 and OKT10 determinants may be expressed by a proportion of T cells undergoing antigen-induced activation in vitro (Kung, et al., 1980) a positive reaction in contact dermatitis with these antibodies was expected. It is possible that antigen-induced transformation in vivo differs from neoplastic transformation of CTCL. This is probably a

quantitative difference rather than an absolute difference in expression of the OKT9 and OKT10 antigens since OKT9 antigen was demonstrated on some lymphoid cells in one other study of allergic contact dermatitis, although a negative reaction was obtained with OKT10 as in this study (Ralfkiaer and Wantzin, 1984). The differences observed in various studies may reflect the sensitivity of the techniques used, and possibly a more sensitive technique such as the avidin-biotin method would produce OKT9 and OKT10 positivity. Most of the blastic transformation of T lymphocytes in contact sensitivity appears to occur in the local draining lymph node (Dahl, 1981) and so it is possible that OKT9 and OKT10 reactivity might be found in nodes draining affected areas. It is, of course, also possible that OKT9 and OKT10 reactivity might be more prevalent at other stages in the contact reaction. Despite these anatomic and technical considerations, the results are compatible with the suggestion that "immature" markers may be preferentially expressed in lymphomas (Chapter 5,6). Comparitive studies of OKT9, OKT10 reactivity in CTCL and lymphomatoid contact dermatitis (Orbaneja, et al., 1976) would obviously be of interest.

The result obtained with OKT6 is also of interest in the context of cutaneous T cell lymphoma (CTCL) where an OKT6+ dendritic subpopulation is present throughout the helper T cell infiltrates (Chapter 5). Contact allergens (Fischmann, et al., 1979; Cohen, et al., 1980) and antigen persistence (Tan, et al., 1974) have been implicated in the pathogenesis of CTCL. The similarity of phenotypes noted here, namely Leu1+, T11+, Leu3a+ with interspersed OKT6+ cells is consistent with this hypothesis and a similar phenotype has also been shown in prelymphomatous states (Chapter 5).

The negative reaction with J5 which detects common acute lymphoblastic leukaemia antigen (CALLA) provides

additional data on the spectrum of reactivity of this marker. A negative reaction was also found in non-malignant lymphoid tissue (this chapter). CALLA is presently known to be expressed on acute lymphoblastic leukemia (ALL) cells (Metzgar, et al., 1981) some lymphomas, (Metzgar, et al., 1981) including CTCL (Chapter 5) and some non-malignant tissues (Metzgar, et al., 1981).

Reactivity of the leukaemia-lymphoma antibodies Be1, Be2 was not readily demonstrated (Be1 positive in 10% of cells in 1 of 4 cases, Be2 negative 3 of 3 cases.) However, similar reservations are required as with the immaturity markers OKT9 and OKT10 (Pages 102-103). In addition, the number of cases tested is small. Reactivity of these markers with other benign dermatoses and lymphoma will be discussed in later chapters.

CHAPTER FIVE

LARGE PLAQUE PARAPSORIASIS AND CUTANEOUS T CELL LYMPHOMA (MYCOSIS FUNGOIDES/SEZARY SYNDROME)

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CHAPTER FIVE

LARGE PLAQUE PARAPSORIASIS AND CUTANEOUS T CELL LYMPHOMA (MYCOSIS FUNGOIDES/SEZARY SYNDROM)

A. INTRODUCTION

The known transition of a percentage of cases of large plaque (atrophic) parapsoriasis (LPAP) and poikiloderma vasculare et atrophicans into clinicaly evident mycosis fungoides (MF) (Plate 25) (Samman, 1972; Everett, 1978) suggests a relationship between the two conditions. Mycosis fungoides (MF) (Alibert, 1835)/Sezary syndrome SS (Sezary and Bouvrain, 1938) (SS), form part of the spectrum of cutaneous T cell lymphomas (CTCL) (Lutzner, et al., 1975) They are considered neoplastic on the basis of the accumulation of mononuclear cells which is initially most evident in skin (and blood in SS), but which may eventually occur in several organs (Rappaport and Thomas, 1974) and may ultimately be associated with a fatal outcome. Many of the cells constituting these infiltrates have been shown to have abnormal nuclear morphology (Lutzner, et al., 1971) and chromosome content by conventional (Whang-Peng, et al., 1982) and cytophotometric (Van Vloten, et al., 1974) methods. However, more physiological analogies may also be drawn in considering the biology of this condition, as cells resembling mycosis cells have been described when normal lymphocytes are "activated" or "transformed" in vitro by phytohaemagglutinin (Yeckley, et al., 1975). Langerhans' cell lymphocyte apposition (Rowden and Lewis, 1976) has also been noted in a manner analogous to delayed hypersensitivity.

The T cell nature of mycosis fungoides has been established by E rosetting techniques (Edelson, et al., 1973), cytochemical (Sterry, et al., 1980; Chu, et al., 1981), and immunocytochemical methods (Brouet, et al., 1973).

PLATE 25. MYCOSIS FUNGOIDES. PLAQUE STAGE.

The predominantly T cell nature of the lymphocytes composing the infiltrates of LPAP was initially determined by the author in pilot studies [using heteroantiserum and peroxidase anti-peroxidase in conjunction with light microscopy (McMillan, et al., 1982b) and immuno-electron microscopy. (McMillan, et al., 1981c)]. These experiments will not be outlined further, but filled a gap in parapsoriasis studies when compared with the wealth of data already obtained in MF. It was considered that testing of LPAP and MF infiltrates with McAbs might identify subpopulations in infiltrates currently labelled under the inclusive term of "T cell". In vitro functional studies have already demonstrated "helper" activity of cells extracted from tissues involved by MF (Berger, et al., 1979) or Sezary Syndrome (SS) (Broder, et al., 1976) Histologically the infiltrates of MF are polymorphous (Lever and Schaumburg Lever, 1983). This, therefore, raises the question of whether the lymphoid component is heterogenous or a monoclonal population of cells expressing "helper" markers.

One study of lymphoblastic leukaemia has shown that utilization of markers expressed at different stages of thymocyte maturation might permit an assessment of the differentiation status of a T cell tumour (Reinherz, et al., 1980). Similar scrutiny of CTCL from this perspective had not been performed before the experiments described below were performed.

The T cell nature of mycosis fungoides and Sezary Syndrome, the prominent position of the T cell in speculation and experimental work on tumour immunity, and the similarities between mycosis cells and transformed lymphocytes have all hampered any search for possible host reactive lymphocytes in the cutaneous T cell lymphomas. However, evidence exists that cells other than classic T cells may be significantly involved in host surveillance.

For instance, athymic nude or neonataly thymectomized mice do not have an especially high incidence of spontaneous or carcinogen-induced tumours (Herberman and Ortaldo, 1981). Natural killer (NK) cells are lymphocytes from normal non-immune donors with the capacity to rapidly lyse tumour cells, virally infected cells, or certain undifferentiated normal cell types in vivo and in vitro (Roder and Pross, 1981). Animal work suggests that NK cells partly fill this gap in tumour surveillance (Herberman and Ortaldo, 1981). The role of NK cells in spontaneously occurring human tumours is less clear. Indirect evidence for such a role could initially be obtained by the demonstration that NK cells accumulate at the site of human tumour development. This subject has recently been reviewed (Roder and Pross, 1981). The available animal evidence suggests that lymhomas would be an ideal subject for initial study. Studies of this kind have been hampered by the lack of a relatively specific immunologic marker for K/NK cells which could be utilized for their in situ identification. Certain McAbs react with K/NK cells, but their cross-reactivity with other lymphoid or myeloid cells impairs their usefulness. Recently a McAb, HNK1 (Leu7) has been produced against human granular lymphocytes with K/NK function (Abo and Balch, 1981). This suggests that studies to detect host immune cells in situ might be feasible.

B. MATERIALS AND METHODS

Twenty-seven cases of CTCL (24 MF, 3 SS), seventeen cases of LPAP (poikilodermatous), and nine cases of chronic benign dermatosis (CBD) (4 lichen planus, LP; 3 atopic dermatitis, AD; and 2 chronic dermatitis of unspecified type) were studied using the method outlined in materials and methods (Chapter 3).

In MF, SS, and LPAP diagnosis was based on clinical criteria and histopathologic evaluation of multiple skin

biopsies (McDonald, 1982; McMillan and Everett, 1982a) (confirmed by authour). Serial sections were examined in all cases classified as LPAP. The MF patients demonstrated similar histopathologic changes (with the exception of one case); i.e., epidermotropic lymphoid infiltrates showing single cell exocytosis and/or Pautrier microabscesses (Plate 26) and consisting of a mixture of small and medium-sized lymphocytes and numerous atypical lymphoid cells with hyperchromatic convoluted nuclei. None of the MF patients had extracutaneous disease at the time of the initial diagnosis as judged by routine staging procedures including chest x-ray, lymphangiography, blood and bone marrow examination, liver biopsy and lymph node biopsy. Thus, other types of T cell leukaemia/lymphoma (eg. T cell chronic lymphocytic leukaemia, node based peripheral T zone lymphoma) with secondary cutaneous infiltrates are excluded.

The one patient with different clinico-pathological features presented with rapid development of large cell lymphoma (LCL) of non-epidermotropic type and poikiloderma after a 2 year history of pruritus. Initial biopsies over the first 2 years showed a superficial perivascular lymphocytic infiltrate. Convoluted or MF cells were conspicuous by their absence in the lymphoma which ultimately developed, despite multiple biopsies from various sites and at various times.

The McAbs used included OKT3/Leu1; OKT4/Leu3A; OKT8/Leu2A; OKT6; OKT9; OKT10; B1/B2; J5; HNK1; LeuM1; LeuM3; R423 and Be1/Be2. The spectrum of reactivity of these antibodies is given in Chapter 3. This battery of McAbs detects mature T cells and subsets (OKT3/Leu1, OKT4/Leu3A, OKT8/Leu2a), immature thymocytes (OKT6, OKT9, OKT10), B cells (B1, B2) other subpopulations such as killer/natural killer cells (HNK1), and monocytes/macrophages (LeuM1, LeuM3). The use of J5 permits comparison with common acute lymphoblastic leukaemia. The

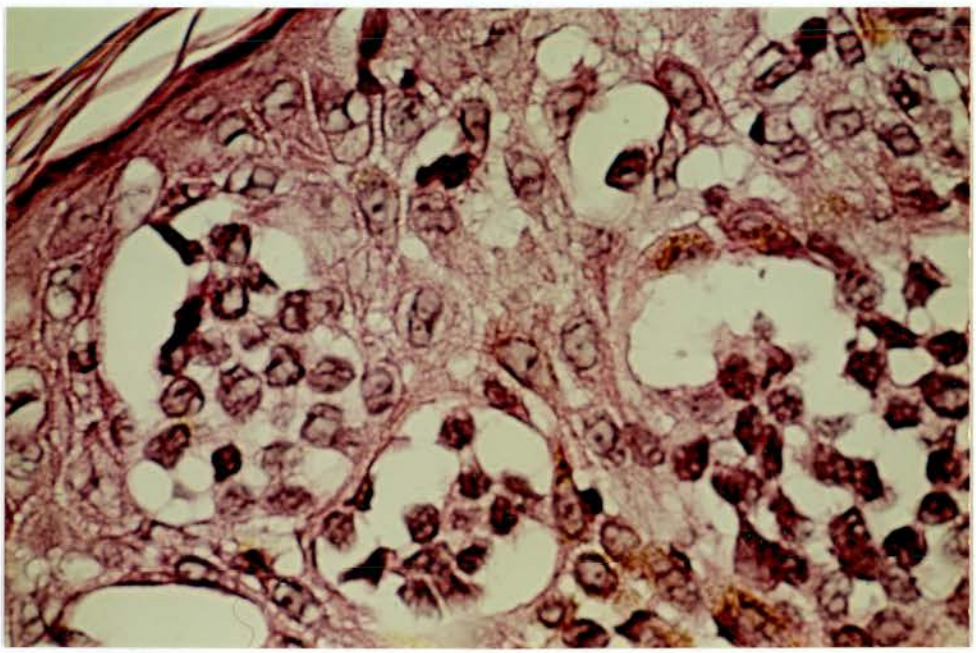


PLATE 26. **MYCOSIS FUNGOIDES.** Epidermal Pautrier microabscesses. Haematoxylin and eosin x 160.

use of LeuM1 further defines the T cell neoplasm since some activated T cells exhibit LeuM1 positivity (Hanjan, et al., 1982). The McAbs Be1 and Be2 are purported to be relatively specific for malignant cells and may be of diagnostic use in peripheral blood studies of CTCL (Berger, et al., 1982). Their utility on tissue sections requires to be determined.

C. RESULTS

The results are summarized in Table 6.

1. MATURE PAN T MARKERS (Leu1/OKT3).

In 20 0f 23 MF the phenotype of exocytic and dermal cells is that of a mature T cell (Leu1+, OKT3+; >50-80% of cells). In 3 cases, all tumour stage, (numbers 2, 12, 15) Leu1/OKT3 stains <50% of cells and in one plaque stage (number 5) although Leu1/OKT3 stains >50% of the total population sheets of negatively staining tumour cells are noted (Plate 27) (which do however stain with OKT9). An OKT3+, Leu1+ phenotype is also noted in SS (60-80% of cells), LPAP (60-90% of cells) (Plate 28, 29) and BCD (65-90% of cells).

Four cases of MF show staining of the majority of cells in Pautrier microabscesses with Leu1/OKT3.

2. SUBSET MARKERS (Leu2A/OKT8).

A helper phenotype is demonstrated in 27/27 CTCL (24 MF; 3 SS) (Plate 30), 14/17 LPAP (cases 4, 15, 17 are exceptions), and 7/9 BCD. There is considerable overlap in H/S ratio among CTCL, LPAP, and BCD, (Tables 6 and 7) (see also results in contact dermatitis). Single exocytic Leu3A+ and Leu2A+ cells (Plate 31) are noted in CTCL, LPAP and BCD. The Pautrier microabscesses in MF show a large number of Leu3A+/OKT4+ cells (Plate 32) but are Leu2A/OKT8- (Plate 31). Leu2A+/OKT8+ cells in MF are found scattered diffusely throughout the dermal infiltrates and occasionally at the

TABLE 6-PROPORTION OF CELLS BEARING MONOCLONAL DETERMINANTS IN CUTANEOUS T CELL LYMPHOMA LARGE PLAQUE PARAPSORIASIS AND BENIGN CHRONIC DERMATOSES

	Case	H/S	Stage	OKT6	J5	OKT9	OKT10	B1/B2	M1/M3
Mycosis Fungoides (MF)									
	1	5:1	P	15%	Nt	±	Nt	0%	Nt
	2	3:1	T	5%	Nt	30%	10%	Nt	Nt
	3	4:1	P	10%	10%	10%	0%	0%	2:1
	4	3:1	P	10%	0%	20%	0%	0%	Nt
	5	5:1	P	20%	0%	40%	20%	0%	1:3
	6	5:1	P	10%	Nt	10%	20%	0%	2:1
	7	4:1	P	20%	Nt	10%	0%	0%	Nt
	8	2.5:1	P	20%	Nt	30%	Nt	Nt	Nt
	9	4:1	P	10%	Nt	30%	0%	0%	Nt
	10	3:1	P	10%	0%	10%	±	Nt	2:1
	11	5:1	P	10%	0%	20%	Nt	Nt	3:1
	12	3:1	T	±	0%	10%	±	0%	Nt
	13a	3:1	P	10%	0%	±	10%	0%	1:2
	13b	3:1	T	Nt	Nt	30%	Nt	0%	0:2
	14	3:1	P	20%	Nt	20%	0%	0%	1:1
	15	2:1	T	±	Nt	±	0%	0%	1:1
	16	4:1	P	±	40%	±	0%	10%(B1)	O:1
	17	2:1	P	±	Nt	Nt	Nt	Nt	Nt
	18	2:1	P	Nt	Nt	Nt	Nt	Nt	Nt
	19	2:1	P	Nt	Nt	Nt	Nt	Nt	Nt
	20	4:1	T	10%	10%	30%	20%	Nt	2:1
	21	3:1	P	10%	Nt	10%	10%	Nt	Nt
	22	3:1	P	10%	Nt	30%	Nt	Nt	1:2
	23	4:1	P	10%	Nt	40%	Nt	0%	0:1
	24	3:1	P	Nt	Nt	10%	Nt	Nt	Nt
	Range	2:1-5:1		0-20%	0-40%	0-40%	0-20%	0-10%	0:1-1:3
No. Pos/No.tested	24/24	H:S>1		17/21	3/9	18/21	6/15	1/13	10/13 M1 13/13 M3
Sezary Syndrome (SS)									
	1	1.5:1		10%	Nt	±	10%	Nt	Nt
	2	4:1		10%	Nt	10%	0%	0%	Nt
	3	4:1		10%	Nt	40%	0%	0%	Nt
	Range	1.5:1-4:1		10%	Nt	0-40%	0-10%	0%	Nt
No. Pos/No. tested	3/3	H:S>1		3/3	Nt	2/3	1/3	0/2	Nt

Key: Nt=Not Tested
±= Equivocal Reaction

TABLE 6 Cont. - Large Plaque Atrophic Parapsoriasis (LPAP)

	Case	H/S	OKT6	J5	OKT9	OKT10	B1/B2	M1/M3
	1	2:1	30%	Nt	10%	0%	Nt	Nt
	2	4:1	30%	Nt	0%	0%	Nt	Nt
	3	3:1	Nt	Nt	0%	0%	Nt	Nt
	4	1:2	Nt	Nt	0%	0%	Nt	Nt
	5	3:1	20%	Nt	0%	0%	Nt	Nt
	6	2:1	±	Nt	0%	0%	Nt	Nt
	7	2:1	10%	Nt	0%	0%	Nt	Nt
	8	4:1	10%	Nt	0%	0%	0%	Nt
	9	10:1	30%	Nt	0%	0%	Nt	Nt
	10	3:1	±	Nt	0%	Nt	Nt	Nt
	11	2:1	30%	Nt	0%	0%	0%	Nt
	12	2:1	20%	Nt	0%	0%	10% (B1)	1:3
	13	2:1	10%	Nt	±	0%	0%	Nt
	14	1.5:1	10%	Nt	<10%	Nt	0%	2:3
	15	1:1	10%	Nt	0%	0%	0%	Nt
	16	3:1	10%	Nt	0%	0%	0%	Nt
	17	1:1	20%	Nt	0%	Nt	0%	Nt
	Range	1:2-10::1	0-30%	Nt	0-10%	0%	0-10%	1:3-2:3
No. Pos/No. tested	14	H/S>1	12/14	Nt	2/17	0/14	1/8	2/2 M1
		H/S≤1						2/2 M3
			Controls					
	LP 1	4:1	±	Nt	Nt	Nt	Nt	Nt
	LP 2	4:1	±	Nt	Nt	Nt	Nt	Nt
	LP 3	1:1	20%	Nt	±	0%	Nt	0:1
	LP 4	2:1	10%	Nt	10%	Nt	0%	2:1
	AD 5	2:1	10%	Nt	0%	Nt	Nt	1:2
	AD 6	2:1	10%	Nt	0%	Nt	Nt	1:1
	AD 7	1:1	10%	Nt	0%	Nt	Nt	1:1
	CD 8	3:1	10%	Nt	Nt	Nt	Nt	Nt
	CD 9	3:1	Nt	Nt	Nt	Nt	Nt	Nt
	Range	H/S<1-4:1	0-20%	Nt	0-10%	0%	0%	2:1-0:1
No. Pos/No. tested	H/S>1	IN 7/9	6/8	Nt	1/5	0/1	0/1	4/5 M1
								5/5 M3

Key: Nt = Not Tested
± = Equivocal Reaction

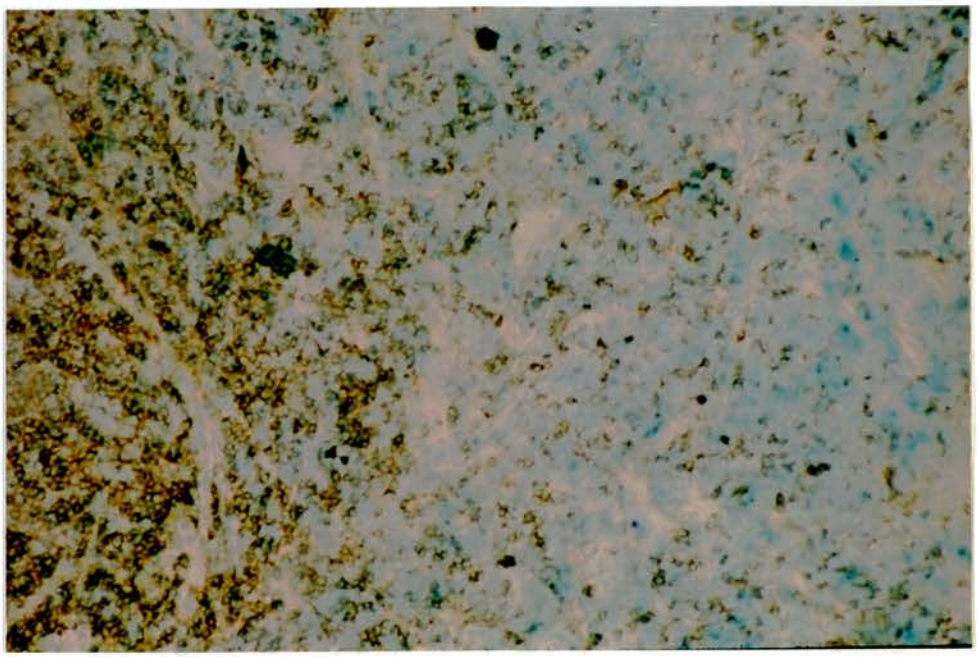


PLATE 27. **MYCOSIS FUNGOIDES.** Mature T cell marker, OKT3, stains numerous cells left 1/3 of field whereas majority of neoplastic population right 2/3 of field stains negatively. Indirect immnoperoxidase. Haematoxylin counterstain x 40.

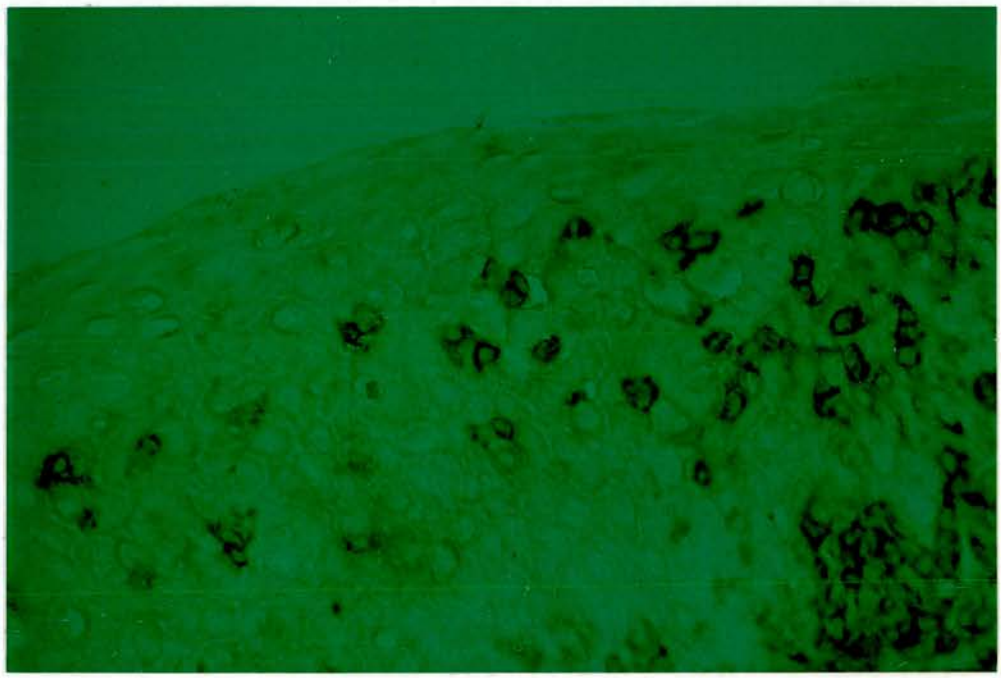


PLATE 28. **LARGE PLAQUE PARAPSORIASIS.** Exocytic Leu1+ T cells. Indirect immunoperoxidase. Non counterstained. Green filter x 100.

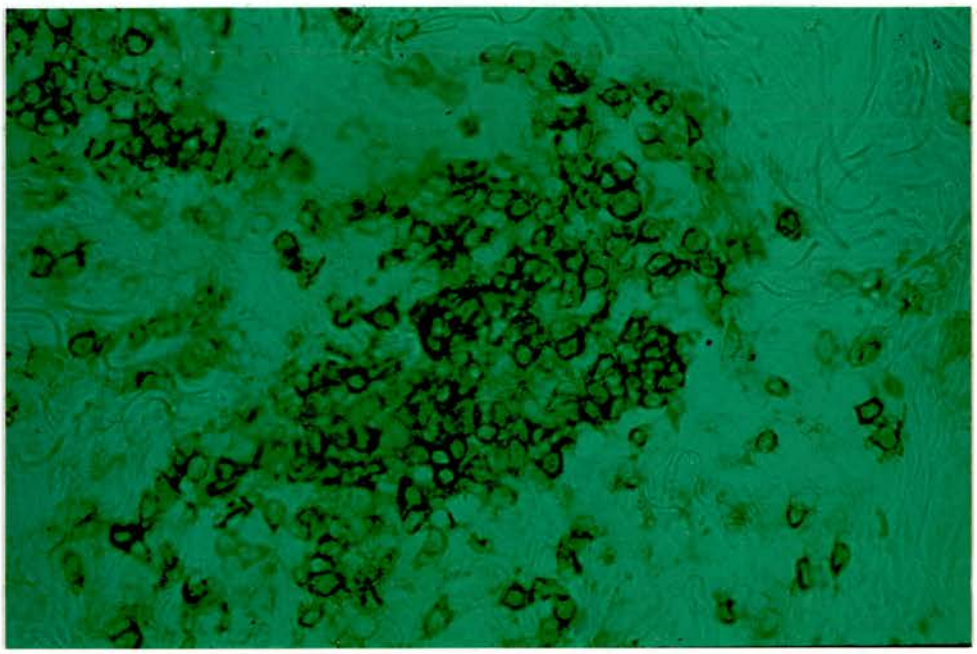


PLATE 29. **LARGE PLAQUE PARAPSORIASIS.** Dermal Leu1+ T cell population. Indirect immunoperoxidase. Non counterstained. Green filter x 100.

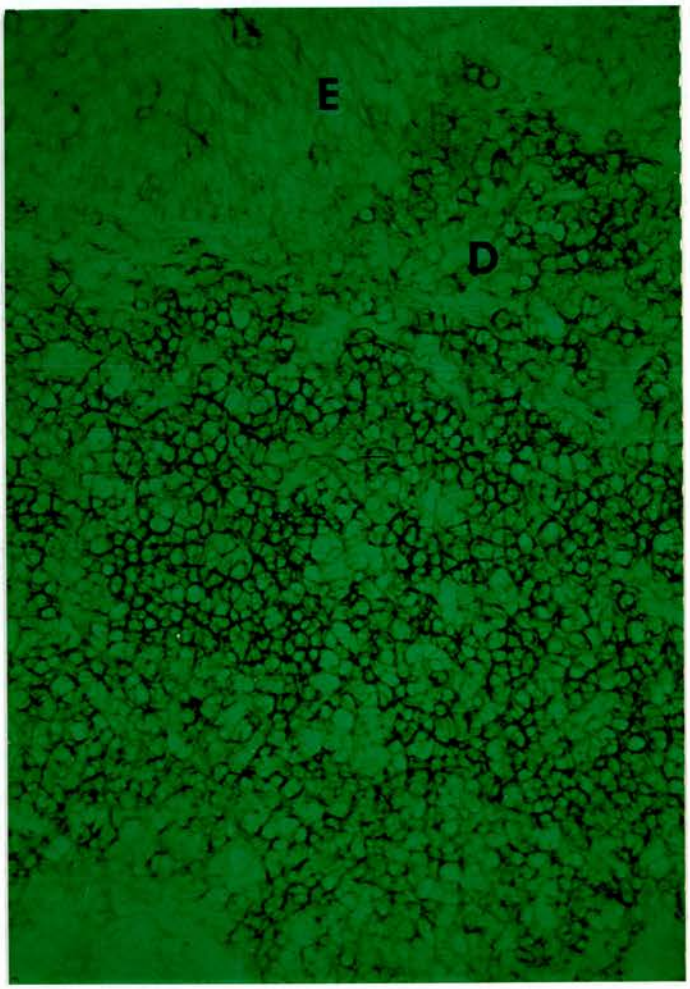


PLATE 30. **MYCOSIS FUNGOIDES.** Dermal Leu3A+ (T helper) cells. Epidermis (E). Dermis (D). Indirect immnoperoxidase. Non counterstained. Green filter x 64.

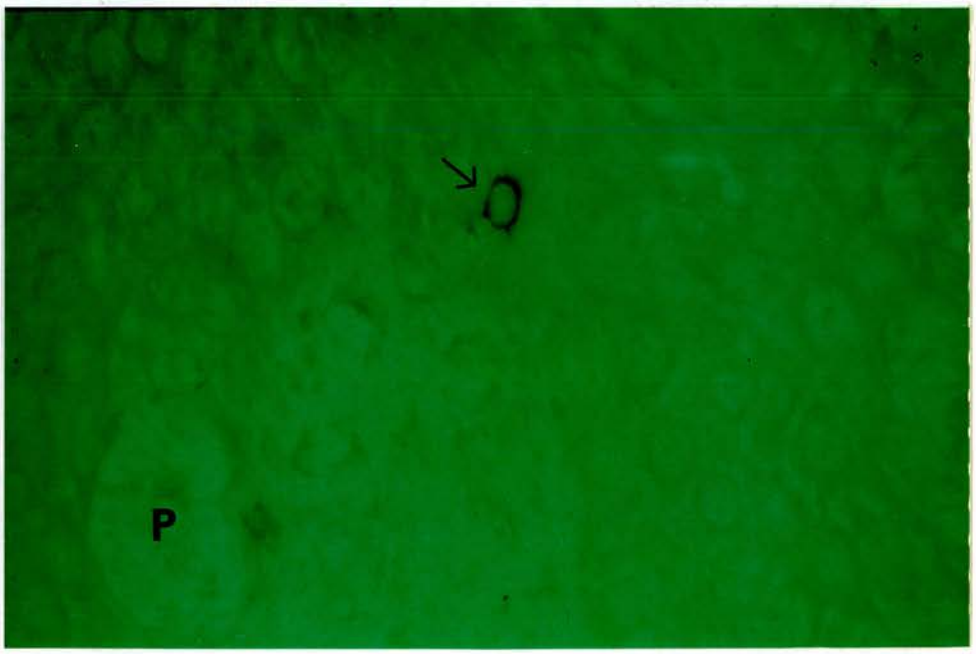


PLATE 31. **MYCOSIS FUNGOIDES.** Isolated epidermal Leu2A+ (T suppressor) cell (arrow). Pautrier microabscess (P) stains negatively with Leu2A, in contrast to Leu3A (Plate 32). Indirect Immunoperoxidase. Non counterstained. Green filter x 160.

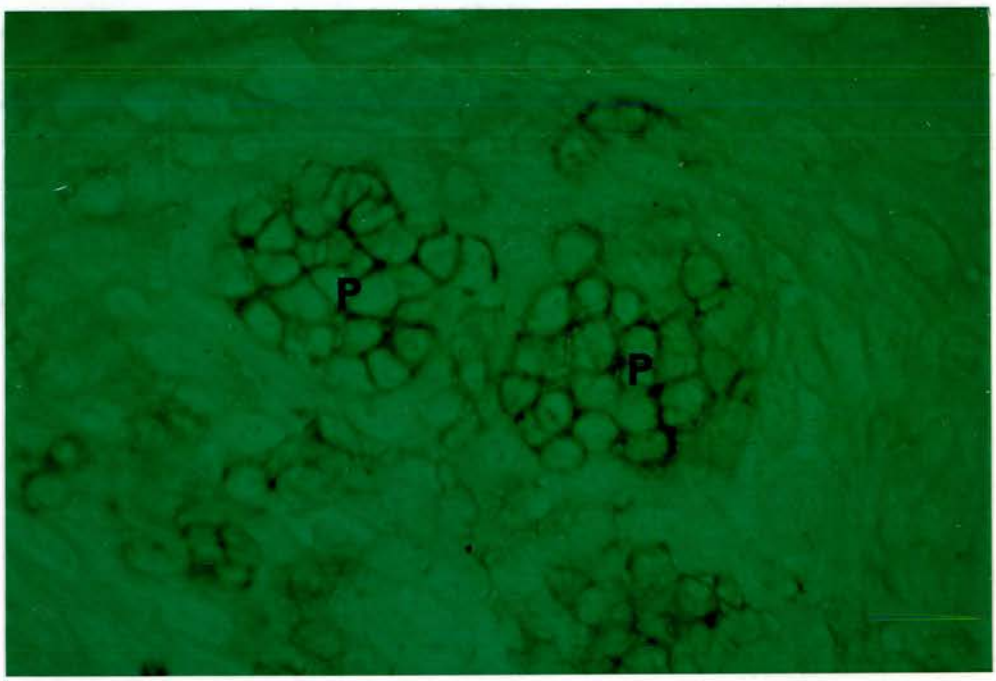


PLATE 32. **MYCOSIS FUNGOIDES.** Leu3A+ (helper T) cells within Pautrier microabscesses (P). Reaction with Leu2A (T suppressor) antibody negative. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

periphery of infiltrates in the reticular dermis. Leu3A+/OKT4+ cells in contrast occur in sheets and clusters.

In 2 cases of MF exocytic cells are OKT3+, Leu1+ but Leu3A-/Leu2A-. In 2 cases of parapsoriasis where exocytic cells are plentiful these are almost entirely Leu2A+/OKT8+.

3. OKT6.

OKT6 is found to react with epidermal dendritic cells in all specimens examined. (Plate 33). OKT6 dendritic cells form a prominent component of Pautrier microabscesses in MF (Plate 34). Dermal OKT6 reactivity (Plate 35) is noted in 17/21 MF, (equivocal in 4), 3/3 SS, 13/15 LPAP (2 equivocal), 6/8 BCD (2 LP equivocal). This result contrasts with the negative results obtained with another dendritic cell antibody R423, which is associated with B cell zones of lymphoid tissue (Chapter 4), benign (Chapter 7), and malignant (Chapter 6) cutaneous B cell infiltrates.

4. J5.

J5 positive lymphoid cells are identified in 3/9 MF. J5 reactivity is not tested in the other conditions mentioned.

5. OKT9.

OKT9 reactivity is observed in 18/21 MF, (10-40% of cells), 2/3 SS, i.e., 20/24 CTCL; but only 2/17 LPAP ($\leq$10% of cells), and 1/5 BCD (10% of cells in LP). See also contact dermatitis results (Chapter 4) for contrast with CTCL. (13 positive patch tests OKT9 negative).

In MF OKT9 reactive cells are intraepidermal (Plate 36), found at the dermo-epidermal junction and intradermal in location. In the latter situation they are found singly and in clusters (Plate 37).

6. OKT10.

OKT10 positive dermal cells (Plate 38) are identified in 6/15 MF (10%-20% of cells), 1/3 SS (10% of cells), i.e., 7/18 CTCL; 0/15 LPAP, 0/1 LP. See also results in contact

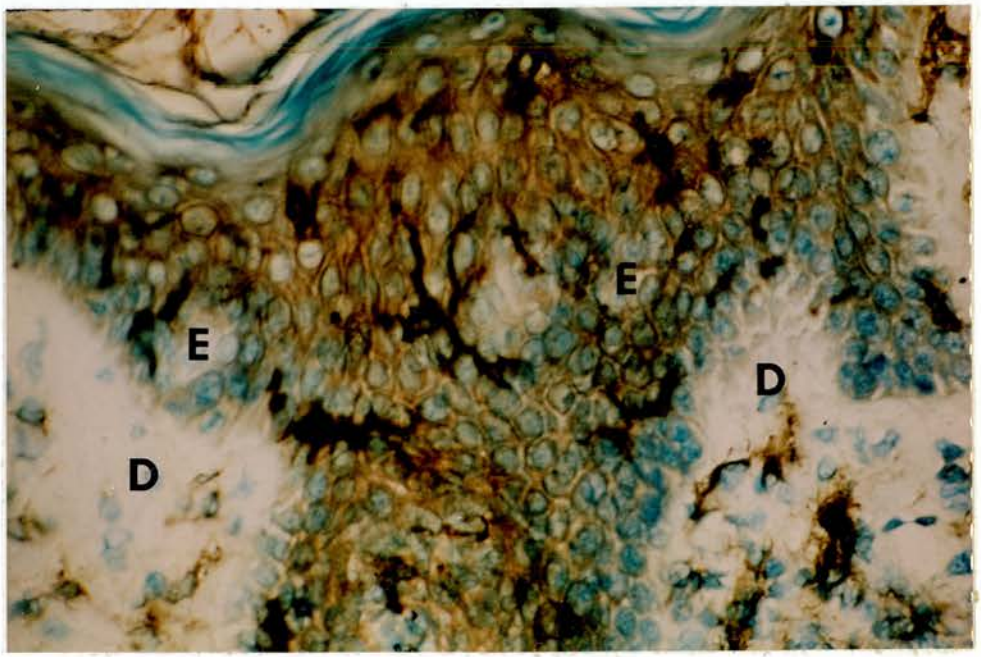


PLATE 33. **MYCOSIS FUNGOIDES.** OKT6+ epidermal and dermal dendritic cells. Epidermis (E). Dermis (D). Indirect immunoperoxidase. Haematoxylin counterstain x 100.

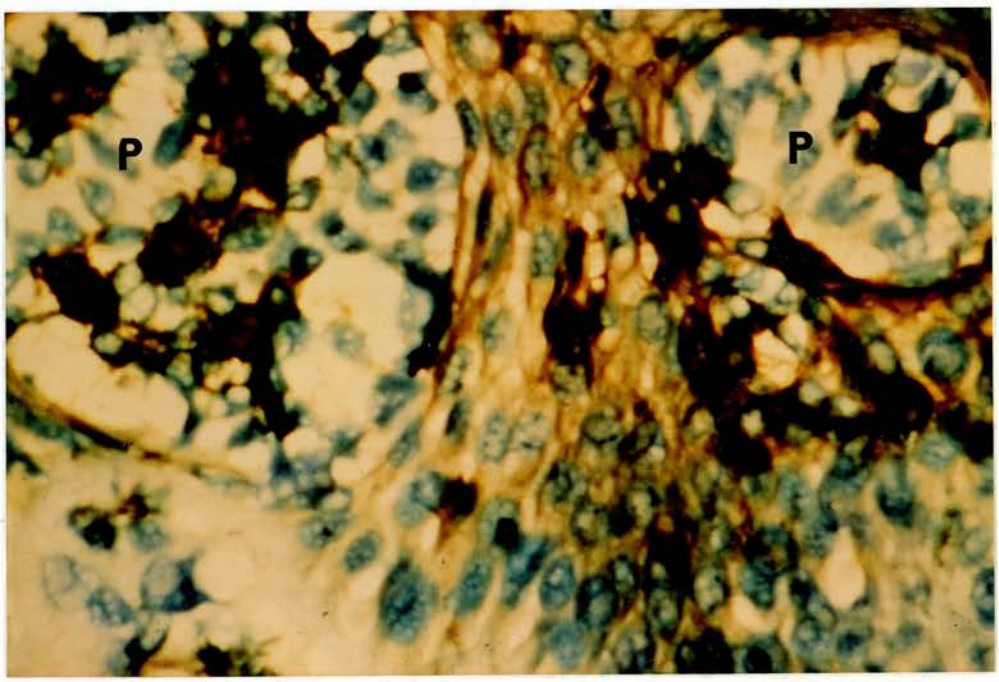


PLATE 34. **MYCOSIS FUNGOIDES.** OKT6+ dendritic cells within Pautrier microabscesses (P). Indirect immunoperoxidase. Haematoxylin counterstain x 160.

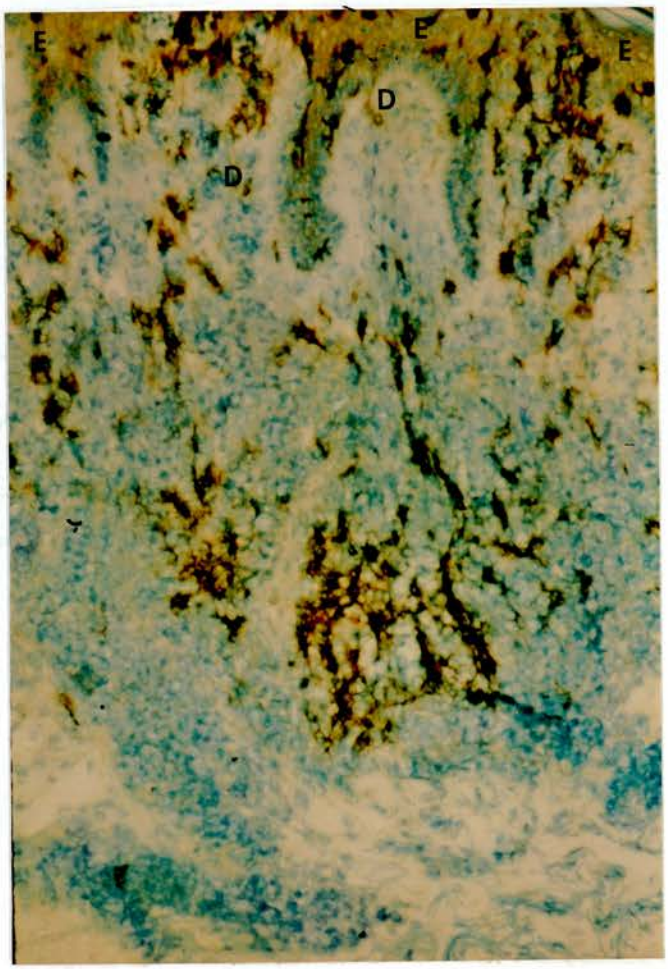


PLATE 35. **MYCOSIS FUNGOIDES.** Dermal OKT6+ dendritic population. Right of centre the OKT6+ cells appear to form a chain. Surrounding lymphoid cells stain negatively. Epidermis (E). Dermis (D). Indirect immunoperoxidase. Haematoxylin counterstain x 40.

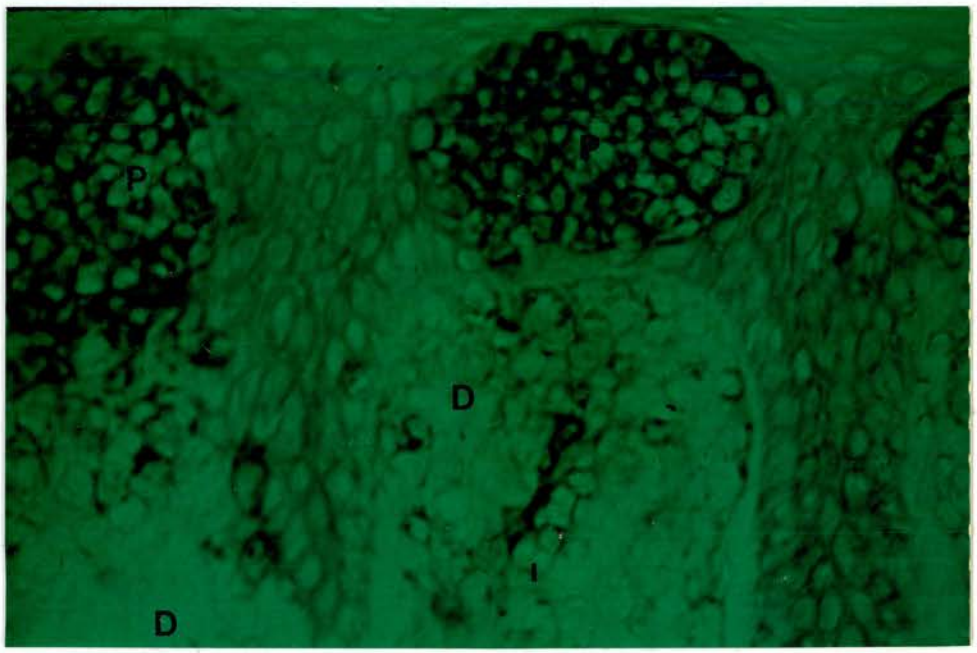


PLATE 36. **MYCOSIS FUNGOIDES.** Pautrier microabscesses (P) contain numerous OKT9+ (immature T/transferrin receptor+) cells whereas tumour population in subjacent papillary dermis (D) contains only rare positively staining cells. Indirect immunoperoxidase. Non counterstained. Green filter x 100.

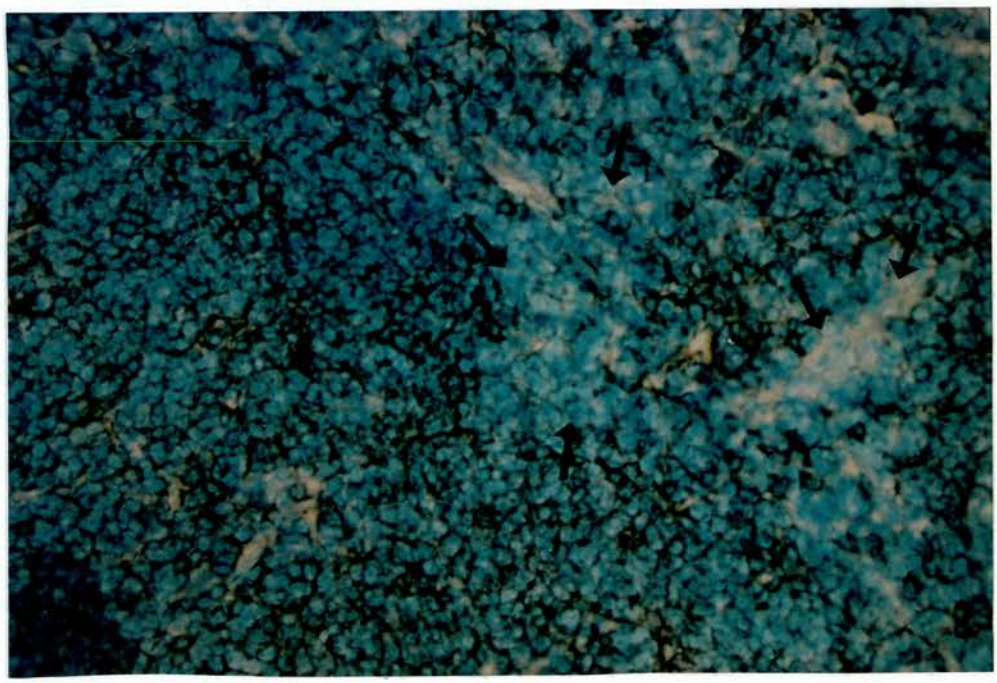


PLATE 37. **MYCOSIS FUNGOIDES.** Dermal OKT9+ population. Negatively staining sheets of tumour cells are present in right half of field (arrow). Indirect immunoperoxidase. Haematoxylin counterstain x 64.

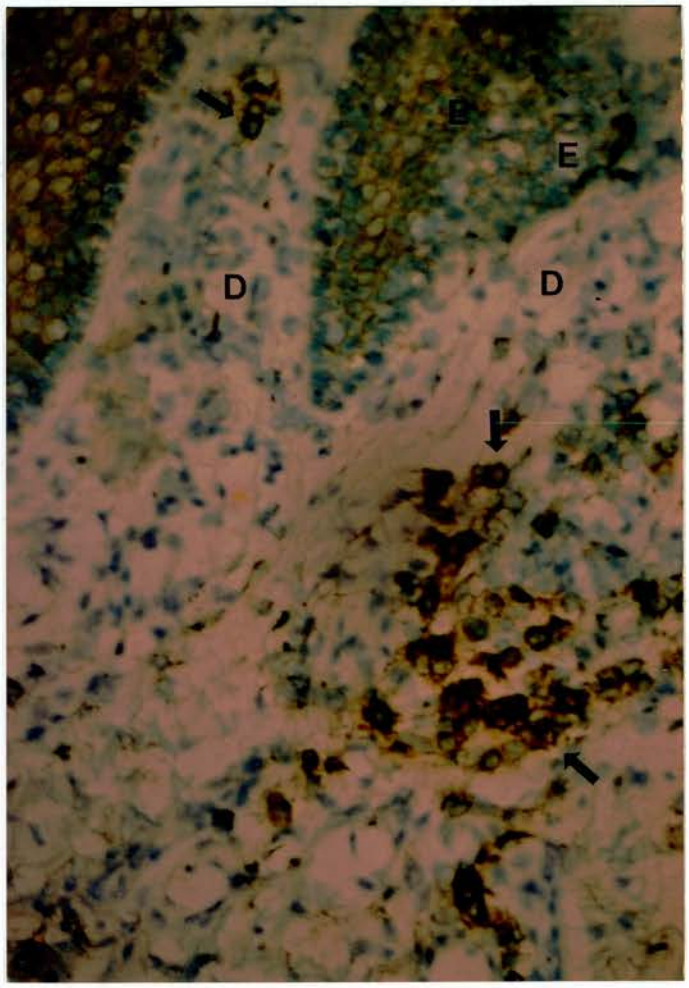


PLATE 38. **MYCOSIS FUNGOIDES.** OKT10+ (immature thymocyte) dermal cells (arrows). Epidermis (E). Dermis (D). Indirect immunoperoxidase. Haematoxylin counterstain x 64.

dermatitis (Chapter 4) for contrast with CTCL. (13 positive patch tests OKT10 negative.)

OKT10 positive cells are not identified in the epidermis.

7. B1/B2.

B cells are identified by reactivity with B1 in 1/14 MF (10% of cells), 0/2 SS, 1/8 LPAP, and 0/1 BCD. See also contact dermatitis, Chapter 4. B1+ cells are dermal in location. No B1+ cells are identified in the epidermis, which contrasts with the mature T cell and subset marker pattern.

No immature B cells (B2+) are identified. This contrasts with results in B cell lymphomas, Chapter 6.

8. LeuM1/M3; Esterase Positive Macrophages.

A monocyte/macrophage component (LeuM1+ and/or LeuM3+) is identified (Plate 39) in 13/13 MF, 2/2 LPAP, and 5/5 BCD. These do not exceed 15% of the cell population in any case. Large esterase positive cells are identified in 21/21 CTCL (18/18 MF, 3/3 SS; 5-15% of cells), 9/9 LPAP (5-15% of cells), 2/3 LP (5-10% of cells).

9. HNK1+ CELLS (Not Shown in Table.)

HNK1+ lymphocytes (Plate 40) are identified in 8/18 MF ($\leq$1%-5% of cells), 3/3 SS ($\leq$1% of cells), 2/9 LPAP ($\leq$1% of cells), 2/3 LP ($\leq$1% of cells), 0/3 AD. HNK1+ cells are usually dermal in location, occupying the papillary and upper reticular dermis. Epidermal exocytic HNK1+ cells are also observed in epidermotropic infiltrates; eg., 4 of 11 CTCL. In one case of MF HNK1+ lymphocytes are seen adjacent to Pautrier microabscesses. Otherwise, they are found scattered in small numbers throughout the epidermis with no apparent clustering effect. However, they are seen in occasional juxtaposition to exocytic T lymphocytes. In the positively staining cases of lichen planus HNK1+ lymphocytes are located at the dermo-epidermal junction as well as

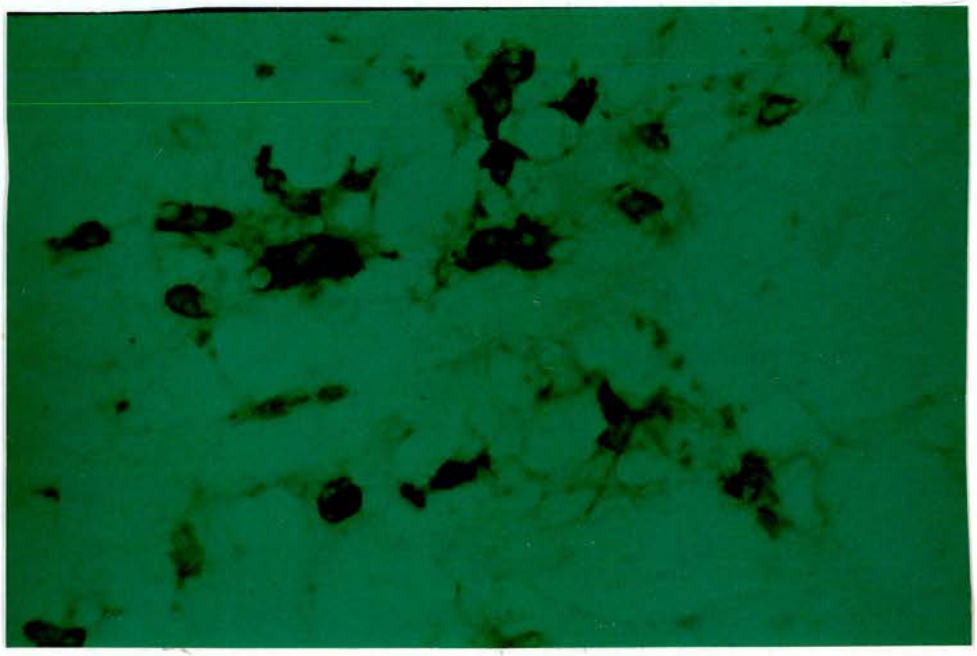


PLATE 39. **MYCOSIS FUNGOIDES.** LeuM1+ dermal cells. Indirect Immunoperoxidase. Non counterstained. Green filter x 160.

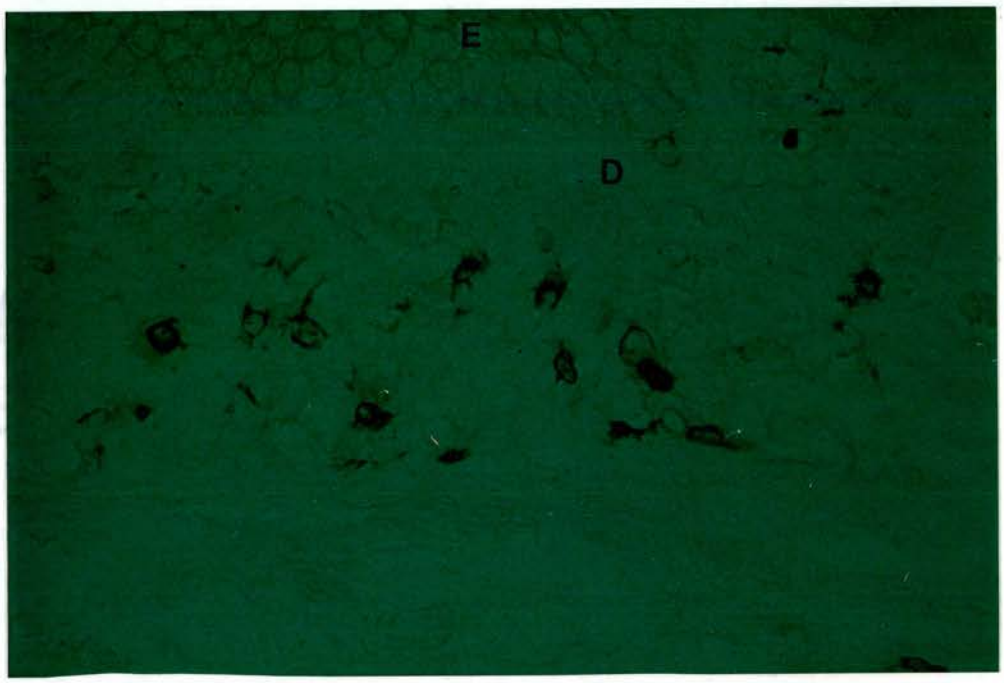


PLATE 40. **SEZARY SYNDROME.** HNK1+ (K/NK) dermal lymphoid cells. Epidermis (E). Dermis (D). Indirect immunoperoxidase. Non counterstained. Green filter x 100.

within the epidermis, and in the lichenoid infiltrates of the papillary and the upper reticular dermis. Examination of counterstained sections of positively staining cases show that the HNK1+ lymphocytes are not intravascular but are located in the interstitium of the dermis, at the dermo-epidermal junction or between epidermal keratinocytes.

10. R423.

A negative reaction is obtained with R423 in 14/14 MF, 1/1 SS, 8/8 LPAP, 2/2 Lichen planus, 4/4 chronic dermatitis.

11. Be1/Be2 (Not Shown in Table).

Be1 reactivity is found in 9/11 MF (10-80% of cells), 3/3 SS (5-60% of cells), 1/5 LPAP (10% of cells), 1/5 AD (70% of cells), 1/3 LP (15% of cells).

Be2 reactivity is found in 6/10 MF (10-60% of cells), 3/3 SS (30-70% of cells), 1/5 LPAP (25% of cells) 1/5 AD (40% of cells, weak staining), 2/3 lichen planus (20-40% of cells (Plate 41).

D. DISCUSSION.

1. Mature Pan T and Subset Markers

The results indicate that the majority of cases of CTCL exhibit a mature helper phenotype but with a proportion of cells present bearing cytotoxic/suppressor markers. This is in contrast to cases of T cell ALL, the majority of cases being OKT3-, OKT4-, OKT8- (immature) in one study. (Reinherz, et al., 1980) A similar mature phenotype of CTCL has been demonstrated by other groups from 1981 onwards (McMillan, et al., 1981d; Kung, et al., 1981; Laroche and Bach, 1981; Haynes, et al., 1981; McMillan et al; 1982c; Holden, et al., 1982a; Knowles and Halper, 1982; Haynes, et al., 1982a; Schmitt, et al., 1982; Thomas, et al., 1982; Wood, et al., 1982; Thivolet, et al., 1982; Mackie and Turbitt, 1982; Hashimoto and Iwahara, 1983; Beuchner, et al., 1983; Sheibani, et al., 1983; Hofman, et al., 1983b; Willemze, et al., 1983a and b; Chu, et al., 1984; Ralfkiaer,

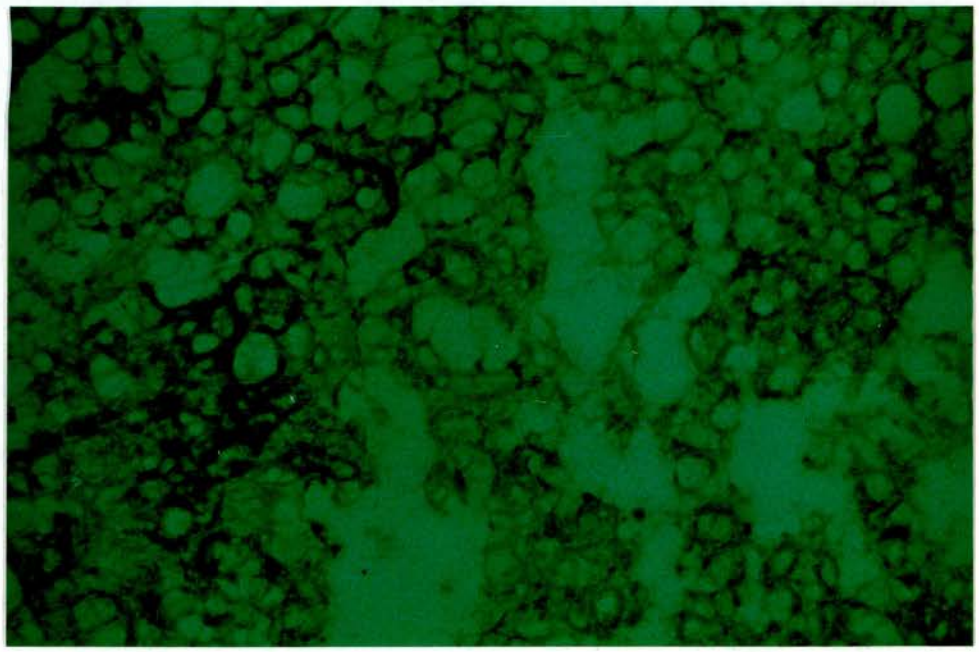


PLATE 41. **LICHEN PLANUS.** Be2+ dermal lymphoid population. Be2 is purportedly relatively specific for lymphoma cells. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

et al., 1985a and b; Nasu, et al., 1985; Abel, et al., 1985; Wood, et al., 1986; Vonderheid, et al., 1987). This result of membrane phenotyping therefore appears to be in agreement with in vitro functional studies. (Broder, et al., 1976; Berger, et al., 1979). Despite this apparent agreement between membrane phenotyping and functional studies in MF it should not automatically be assumed that a phenotype typical of a particular subset implies the same functional activity. For instance, in chronic lymphocytic leukemia (T cell type) cells have been identified which have a "suppressor" phenotype immunologically (Leu2A+) but "helper" function (Siegel, et al., 1981).

This then raises the question of the significance of the cells in MF infiltrates reacting positively with "suppressor" antibodies. Whether similar results would be obtained in skin with pre-existing subset markers (Gupta, et al., 1978) is unknown. The nature of the cells reacting positively with suppressor antibodies is open to speculation. In the majority of cases studied here, the number of cells reacting positively with "helper" and "suppressor" antibodies suggests that these reactivities can be accounted for by separate subpopulations of cells. In 4 cases, however, the number of Leu3A+/OKT4+ cells appears similar to that of Leu1+/OKT3+ cells suggesting that the subset markers (Leu3A+/OKT4+ plusLeu2A+/OKT8+) exceed the Lue1+/OKT3+ component unless some cells are expressing both markers simultaneously. One group has suggested that a subgroup of CTCL does indeed express OKT4/OKT8 simultaneously and that this may be of diagnostic use (Chu, et al., 1984). Their suggestion is based on the observation that "helper" and "suppressor" numbers exceed the quantity of cells detected by pan T cell markers. This could arise from malignant dedifferentiation or by a more physiologic process. Simultaneous expression of markers normally found on separate subpopulations has been described previously on

non-malignant lymphocytes when conventional T and B cell markers were used (Dickler, et al., 1974), and a similar phenomenon may occur with the cell surface markers detected by "helper" and "suppressor" subset antibodies (Bach and Bach, 1981)

This situation does need further clarification as a proportion of macrophages may express Leu3A/OKT4 antigenic determinants (Wood, et al., 1983a), and presumably these cells, which are OKT3-/Leu1-, could account for some of the "subset" numbers exceeding the pan T cell population. Leu1-, Leu3A+, Leu2A- malignant lymphocytes could also contribute to this phenomenon.

This area is further complicated by the demonstration in vitro (Pichler, et al., 1978) that "suppressor" cells may change to "helper" cells. Whether lymphocytes may change their surface markers after migration into the skin is presently unknown.

One study utilizing the more direct approach of double labelling immunofluorescence has failed to demonstrate Leu3A+/Leu2A+ cells in MF (Ralfkiaer, et al., 1985a)

None of the rare cases of MF with a purely suppressor phenotype (Thomas, et al., 1982) were noted in this study. However, one point worthy of consideration is whether the "suppressor" cells identified here immunologically may express "helper" function as in T CLL or whether they exert "suppressor" function and may even exert a controlling effect on the transformation and multiplication of the "helper" cells. "Suppressor" lymphocyte activity has certainly been demonstrated in some patients with Sezary Syndrome (Kansu and Hauptman, 1979). One study (Vonderheid, et al., 1987) indicates that Leu2A+ "suppressor" cells do not correlate with responses to treatment in pretumour MF. The authours concluded that T suppressor cells play little biologic role in the natural history of CTCL. Data should be obtained from other groups before definitive conclusions

are reached.

The architectural pattern of "helper" and "suppressor" cells is similar to that formed in other studies (Willemze, et al., 1983a; Chu, et al., 1984) of CTCL. No case with alignment of OKT8+ cells at the dermo-epidermal junction (separated from OKT4+ dermal lymphocytes), as described by Thomas et al. (Thomas, et al., 1982), is noted. Exocytosis of Leu3A+ cells is more evident in MF when compared to parapsoriasis where exocytic cells tend to be Leu2A+.

Further studies will be required to determine whether cases of LPAP undergo a change of H/S ratio with progression of MF. The overlap of H/S ratios observed between CTCL, LPAP and CBD (Tables 6 and 7) indicates that simple assessment of H/S ratios in the skin would be of limited diagnostic help in MF, and this is in agreement with one other study (Willemze, et al., 1983a).

A loss or deficiency of mature cell markers is noted here, both in focal areas and throughout the cell population. This is more evident in tumour stage MF and aberrant phenotypes have been noted in advanced stages of the disease elsewhere (McMillan, et al., 1982c; Holden, et al., 1982b; Knowles and Halper, 1982; Haynes, et al., 1982a; Schmitt, et al., 1982; Wood, et al., 1982; Willemze, et al., 1983a; Ralfkiaer, et al., 1985a; Nasu, et al., 1985; Abel, et al., 1985; Wood, et al., 1986). One study suggests this change may actually be noted at an early stage and be of diagnostic use (Chu, et al., 1984), but the latter point is presently controversial (Ralfkiaer, et al., 1985a).

The mature phenotype of 20 of 23 cases of MF is compatible with a stem cell disorder (Chapter 2) which still results in more differentiated compartments being larger. However, the 3 tumour stage cases with <50% of cells staining with mature markers and 1 plaque stage with sheets of tumour cells staining negatively with the same reagents indicate that a differentiation block (Chapter 2) may occur

TABLE 7 - COMPARISON OF FINDINGS IN RECENT IMMUNO-PHENOTYPIC STUDIES OF CUTANEOUS T CELL LYMPHOMA

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
1) Present Study	OKT3+ Leu1+ OKT4+ Leu3A+ in 27/27 H/S 2:1-5:1 (24 MF) (3 SS)	20/24 0-20%	3/9 Cases 0--40%	1/16 Cases 10%	7/18 Cases 0-20%	21/21 Esterase 10/13 LeuM1 13/13 LeuM3	4/26 Loss of Leu1 and Leu3A or Leu3A alone	H:S 1:1 (2) H/S 2:1 -4:1 (7) 1/5 T9 positive (10% of cells)	OKT3+ Leu1+ OKT4+ Leu3A+ in 14/17. OKT9 positive cells 2/17 cases	20/24 0-40%	Leu2A+ OKT8+ component diffusely scattered and/or around deep edge of infiltrate See results
2) Kung et al 1981	OKT1+ OKT3- OKT4- in 1/1				1/1		1/1, loss of OKT3, OKT4			1/1	
3) Haynes et al 1981	Blood S/S cells OKT3+ OKT4+ (5/5) SS										

Note: Blank in a column = Not examined in study cited

TABLE 7 CONTINUED

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
4) Haynes et al 1982a	OKT3+ OKT4+ in 11 Cases (10MF)	Present 11/11					2 Cases OKT4- OKT8- in epidermal component 1 case OKT4- OKT8-	OKT4+ (OKT3 variable) in 4DHS		Positive in 11/11	Skin CTCL cells 3A1+ but blood cells 3A1-
5) Haynes et al 1982b								3 Delayed Hyper-sensitivity (DHS) reactions negative for OKT9	OKT9 positive in 1/1	10-70% in 4/5	Phenotype of skin and circulating blood cells differed in HTA1 expression
6) Thomas et al 1982	OKT4+ in 6/7	Yes, in epidermis									OKT4+ cells closely related to dermal Ia+ cells; 1 case showed OKT8+ at DE junction and OKT4+ in dermis

TABLE 7 CONTINUED

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
7) Holden et al 1982a	OKT3+ OKT4+ in 5/5	Epidermal and Dermal location in 5/5 7-18 5% of cells									No Epidermal OKT8+ cells. OKT4+ plus OKT8+ component >100%
8) Holden et al 1982b		Few OKT6 positive cells					Loss of OKT4 OKT8				
9) Knowles and Halper 1982	OKT3+ OKT4+ in skin (1/1) PBL (6/6) LN (8/8)	OKT6-			OKT10-		OKT3+ OKT4+, OKT3+ OKT4- (blood). OKT3- OKT4+, OKT3+ OKT4+ (LN).			Virtually No OKT6+ OKT9+ OKT10+ cells	Variation in intensity of OKT3 OKT4 staining

Note: Blank in a column = Not examined in study cited.

TABLE 7 CONTINUED

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
10) Wood et al 1982	25/25 Leu3A+ Leu4+ (Leu2A+ 5-10% of infiltrate)	Present including Pautriers				1/3 of infiltrate (Detected by alpha-naphthyl butyrate esterase)	2 cases Leu1-				Epidermal cells Leu3-/2- in 3 cases. Leu2A+ scattered. Ia+ and Ia- subtypes also described 3A1 reactivity variable
11) Schmitt et al 1982	OKT3+ OKT4+	Present in 5/5 MF and 4/5 SS					Some blastic OKT4- tumour cells				
12) Mackie and Turbitt 1982				Yes, in some biopsies small nos.							

TABLE 7 CONTINUED

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
13) Sheibani et al 1983	Leu1+ Leu3A+ Leu5+			B1 and BA1 negative 1/1 case							
14) Willemze et al 1983a	OKT3+ OKT4+ Leu1+ Leu3+ in 19 CTCL (16 MF; 3SS)	Considerable overlap noted in skin H/S between CTCL and benign dermatoses (AD, CD, Chronic eczema)		IgM, Kappa or Lambda Component Negligible			Blast like cells present with Leu1- (3) or Leu3A- OKT4- phenotype or Leu1-, Leu3-, OKT4- (4 cases)	5AD showed Leu3A+ OKT4+ phenotype 8 chronic eczema; 1LP; 3CD			OKT4 staining weaker than Leu3A; Leu2A scattered at periphery; Leu3A+ cells in clusters; decreased Leu2A+ cells in advanced cases.
15) Hofman et al 1983b	Leu1+ 70-80% Leu3A+ H70-80% S 5-10% (5 MF 3 SS)	Epidermal and dermal dendritic cells. (20% of dermal infiltrate)						5 LP showed H/S 1:1 H/S 7:1 in 1 AD.			

Note: Blank in a column = Not examined in study cited.

TABLE 7 CONTINUED

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
16) Hashimoto and Iwahara 1983	Leu1+										IEM indicates some convoluted cells not T cells.
17) Chu et al 1984	OKT1+ OKT4+ in 85% OKT1+ OKT4+ OKT8+ in 15% H/S mean 6.5:1				92% of cases had OKT10+ cells (1-20%) No OKT10+ cells in epidermis		Loss of OKT1 in 21% of patients.	OKT1+ OKT4+ phenotype in 5 LP and 1 discoid LE. No OKT10 in benign dermotoses			26% also contained epidermal T cells No direct conparison of H/S in CTCL and benign
18) Nasu et al 1985	T11+ OKT3+ OKT4+ in 14/18 OKT8+ in 1			6/6 HLB3 negative 6/6 SIg negative	MF 11/11 negative SS 6/6 negative		Leu1- in 2. Weak OKT4+ in tumours in 1 MF			10/12 positive <20% (5) to >50% (3) in MF; 6/6 positive S/S <20% (3) to >50% (3)	ABC Method

TABLE 7 CONTINUED

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
19) Abel et al 1985	Leu1+, Leu3A+ Leu4+ Leu5+						Leu8- in 18/22				
20) Ralfkiaer et al 1985	18 Helper 1 Sup-pressor	Yes in all biopies and in Pautrier.			More common in MF than LPAP	No R423+ cells.		No BCD OKT8+ but 8 were 1:1 H/S 27 were Helper	LPAP OKT8+ phenotype 29 Helper	Commoner in MF than LPAP	Overlap between BCD, MF LPAP
21) Wood et al 1986							Leu8, Leu9 deficiency versus controls				Tumour stages aberrant

TABLE 7 CONTINUED

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
22) Turbitt and Mackie 1986					Yes OKT10+ cells commoner in MF		Leu 8 deficiency in MF	Overlap of OKT9 and OKT10 reactivity in BCD and MF		OKT9+ cells more prevalent in MF	
23) Vonderheid et al 1987	Mature Helper							Overlap of H/S ratio between BCD and CTCL			Leu1/Leu4 ≥ 70% and H/S ratio ≥ 6 used as diagnostic criteria of MF
24) Abel et al 1988	Mature T						Leu8/9 deficiency unhelpful discriminant between benign and malignant erythrodermas	Psoriasis, PRP, drug eruptions included			Erythroderma main subject of study

Note: Blank in a column = Not examined in study cited.

TABLE 7 CONTINUED

Study	Phenotype Mature Markers	OKT6 Dendritic Component	J5 Positive Cells	B Cell Component	T10 Positive Cells	Macrophage Component	Loss of Mature Markers	Benign Dermatoses	Large Plaque Para-psoriasis	Transferrin Receptor	Other Comments
25) Oliver and Winkelman 1989	Mature T Helper										Involved Unilesional Mycosis Fungoides

Note: Blank in a column = Not examined in study cited.

in some cases of MF. The 2 cases of MF showing Leu1+ OKT3+ Leu3A- OKT4- Leu2A- OKT8- exocytic cells (phenotype not found during normal intrathymic differentiation, Table 5) imply that aberrant differentiation may also be a concomitant of this disease. The theories of stem cell disorder (single clone expansion) versus differentiation block have been proposed in a mutually exclusive manner to relate malignancies to normal cellular differentiation (McGrath, 1981). The above observations suggest this viewpoint requires modification as all 3 mechanisms of disturbed differentiation outlined in Chapter 2 (differentiation block, stem cell disorder, and aberrant differentiation) may occur in one disease.

2. OKT6.

The finding of OKT6 reactivity with epidermal dendritic cells in the first cases of LPAP and MF studied (McMillan, et al., 1981b; McMillan, et al., 1982d) was somewhat surprising (Plates 42, 43) as this antibody was initially used in an attempt to detect immature thymocytes. (Also see reactivity of OKT6 with dendritic cells in normal skin and crypt epithelium of reactive tonsil, Chapter 4.) This finding was first communicated in 1981 (McMillan, et al., 1981b) and double labelling immunofluorescence (Fithian, et al., 1981) and single labelling EM (Murphy, et al., 1981) studies in two independent laboratories being conducted at the same time confirmed this OKT6 reactivity resided in Langerhans' cells.

OKT6+ cells form a variable proportion of the infiltrates of CTCL, LPAP, and CBD examined. Similar findings have been documented elsewhere (McMillan, et al., 1981b; McMillan, et al., 1981d; McMillan, et al., 1982d; Thomas, et al., 1982; Wood, et al., 1982; Hofman, et al., 1983b; Chu, et al., 1984; Ralfkiaer, et al., 1985a; Wood, et al., 1986). (The findings of OKT6 reactivity in a variety of T cell infiltrates contrast with monoclonal antibody

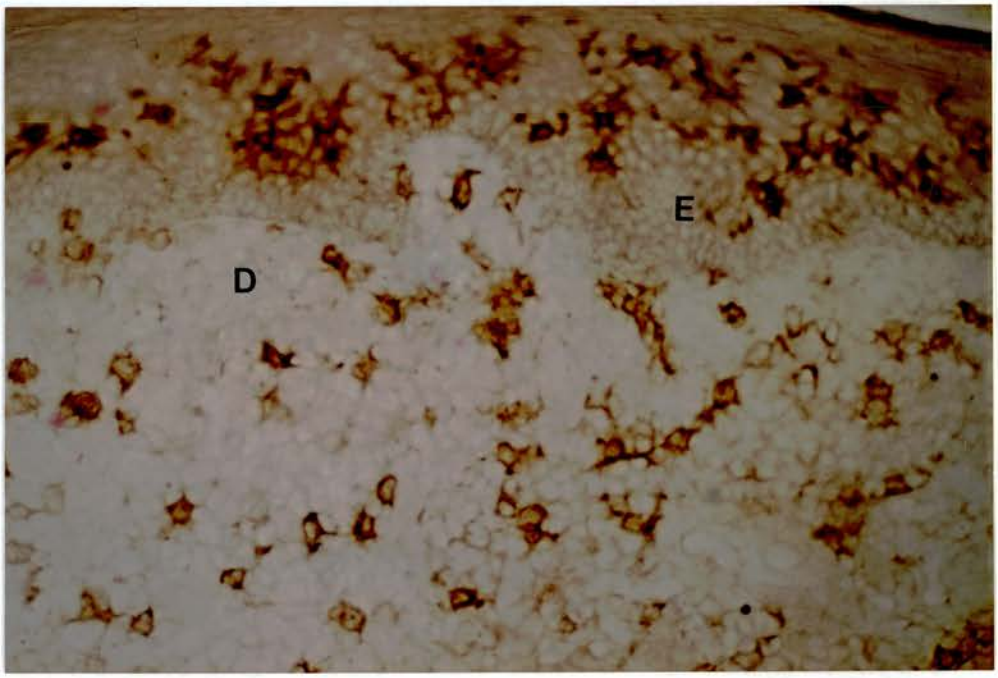


PLATE 42. **LARGE PLAQUE PARAPSORIASIS.** OKT6+ dendritic cells in epidermis (E) and dermis (D). Surrounding lymphoid cells stain negatively. Indirect immunoperoxidase. Non counterstained x 64.

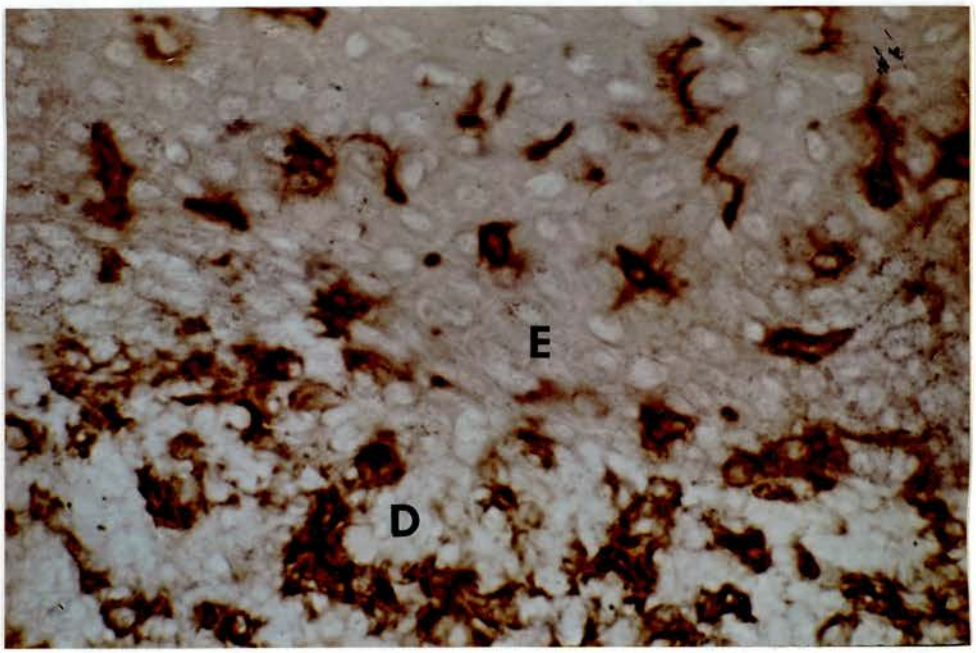


PLATE 43. **LARGE PLAQUE PARAPSORIASIS.** One of the first cases to show cross reactivity of common thymocyte antibody OKT6 with epidermal dendritic cells. Epidermis (E). Dermis (D). Indirect immnoperoxidase. Non counterstained x 160.

R423, another dendritic cell antibody, which produced a negative reaction in all specimens examined.)

OKT6 reactive cells could, therefore, occur in MF for either of two reasons. First the lymphomatous nature of this condition suggests that immature T cells might be present (McMillan, et al., 1981d). The OKT6 marker is expressed on T cells at an intermediate stage of intrathymic differentiation (Reinherz, et al., 1980; Kung, et al., 1980) located in thymic cortex (Bhan, et al., 1980; McMillan. et al., 1982a) (Chapter 4) and a subset of human T cell lymphoblastic lymphoma has been shown to have an OKT6 positive, cortical thymic phenotype (Bernard, et al., 1981). Secondly, cells with the ultrastructural morphology of Langerhans' cells have been described in close apposition to lymphocytes in the cutaneous infiltrates of MF (Rowden, et al., 1979) and hypotheses have been advanced concerning the role of Langerhans' cells and helper T cells in the induction of MF (Cohen, et al., 1980). At least some dermal OKT6 positive cells in CTCL contain Birbeck granules and are, therefore, Langerhans' cells (Holden, et al., 1982c). The Langerhans' cell might be involved in a reactive process against a persistent antigenic stimulus (Tan, et al., 1974)) or may be transformed by a retrovirus (VanDerLoo et al., 1979). In experimental lymphomas histiocytes may induce proliferation of surrounding lymphocytes (Ponzio, et al., 1977). Research is currently underway to determine whether morphologic differences exist between the OKT6 positive cells in benign and malignant infiltrates. The possibility that the OKT6 positive cells in the cutaneous infiltrates of CTCL are immature T cells rather than Langerhans' cells has not been substantiated. However, the circulating OKT6 positive cells in CTCL do appear to be lymphoid rather than histiocytic (Chu, 1983).

3. J5.

The presence of J5 reactivity in a proportion of CTCL

cells (40% in 1 case) is of interest. The number of cases assessed is too small to gain any notion of the frequency of this phenomenon, and no benign infiltrates were tested for comparison. However, the equivocal or negative results obtained in tonsillar tissue (Chapter4) indicate the rarity of expression of this antigen in normal lymphoid tissue. The results obtained here in CTCL indicate that this antigen is not restricted to common acute lymphoblastic leukemia cells and neutrophils but may be found in other malignancies. It's presence has also been identified in lymphoblastic lymphoma (Ritz, et al., 1981). Future comparisons with benign dermatoses are obviously required.

4. OKT9.

The results obtained with OKT9 indicate that a difference exists between CTCL, LPAP, CBD and ACD in their expression of this determinant (20/24 CTCL; 10-40% of cells: 2/17 LPAP; ≤10% of cells; CBD 1/6 cases ≤10% of cells; 0/14 ACD) (Chapter 4). Preferential expression of the transferrin receptor in CTCL has been shown in earlier parts of this project (McMillan, et al., 1982e; McMillan, et al., 1983a) on smaller numbers of patients and similar findings have subsequently been published from other laboratories (Haynes, et al., 1982a; Ralfkiaer, et al., 1985a). However, the overlap with benign infiltrates and the demonstration by one other group of significant numbers of OKT9 reactive cells in allergic contact dermatitis (Ralfkiaer, et al., 1984a) suggests that any diagnostic application would require clinicopathologic correlations. The demonstration of OKT9 reactivity in benign infiltrates (1/5, LP; 10% of cells) is not surprising since some lectin stimulated T cells may be OKT9 positive (Greaves, et al., 1981).

In order to further characterize the OKT9 positive cells in CTCL more information is required concerning the expression of this marker in non-malignant states. For instance, when mature T cells (OKT3+) (Reinherz, et al.,

1979) become activated do they all retain OKT3 positivity (OKT3+, OKT9+) or do they dedifferentiate losing the OKT3 marker (OKT3-/OKT9+)? Likewise what is the phenotype of the OKT9 reactive cells in CTCL? Are they OKT3-/OKT9+ or OKT3+/OKT9+ or are both types of cells present? The presence of sheets of cells in 2 cases of CTCL (1 tumour; 1 plaque) which were OKT3-, Leu1- but OKT9+ indicates the presence of the former phenotype (OKT3-/OKT9+) which is normally found on immature thymocytes (Chapter 4). Studies of cutaneous lymphoma of non MF/ non SS type mentioned later (Chapter 6) indicate the latter phenotype (OKT3+/OKT9+) may at least occur in CTCL of non MF type. Phenotypic comparisons of CTCL cells and activated lymphocytes are all the more intriguing in view of the ultrastructural similarities between CTCL cells and activated lymphocytes (Yeckley, et al., 1975).

Apart from antigenic stimulation of mature OKT3+, Leu1+ T cells or malignant dedifferentiation, OKT9 reactivity may also be present in CTCL simply as a function of lymphoproliferation. The OKT9 determinant is neither T cell nor tumour specific and is found on a wide range of proliferating cell types, eg., fast cycling fraction in T cell ALL, dividing cells in foetal thymus, foetal liver, normal adult bone marrow, and malignant cell lines of teratocarcinoma, mammary carcinoma, and neuroblastoma (Greaves, et al., 1981).

The demonstration that the OKT9 determinant recognizes a transferrin receptor (Sutherland, et al., 1981) raises an additional perspective. DeSousa et al. have provided evidence that disturbed lymphocyte traffic (ecotaxopathy) secondary to upsets in iron metabolism may be involved in the pathogenesis of Hodgkin's disease (DeSousa, et al., 1978) and Rowden et al. (Rowden, et al., 1981) have suggested similar possibilities should be considered in mycosis fungoides. It has previously been suggested that

abnormal lymphocyte traffic might be involved in producing the lymphoid accumulation in MF (Tan, et al., 1974). Further studies of iron metabolism, eg. ferritin, transferrin, and lactoferrin would obviously be of interest.

5. OKT10.

The OKT10 McAb was originally found to react with prothymocytes and some T cell ALL (Reinherz, et al., 1980). In this study differences are found when CTCL (7/18 positive; 10-20% of cells) is compared with LPAP (0/14) and ACD (0/14) (Chapter 4). The results expand our earlier results in a smaller number of cases (McMillan, et al., 1983b; McMillan, et al., 1984). Chu et al. have also demonstrated OKT10 positive cells in CTCL (92% of cases; 1-20% of cells) with negative results in a variety of benign dermatoses (Chu, et al., 1984). Again, although not specific for lymphoma or immature T cells this antibody should be tested in further examples of lymphocytic infiltrate in view of its preferential expression in CTCL.

6. B1/B2.

The monoclonal antibody B1 reacts with a small number of cells in the conditions examined (1/15 CTCL; 1/8 LPAP; 0/1 LP). See also contact dermatitis Chapter 4. Negative results are obtained with B2 which detects immature B cells. These results taken in conjunction with those using pan T cell markers confirm the T cell nature of CTCL, LPAP, and LP. Of the large number of recent McAB studies examining CTCL (McMillan, et al., 1981d; Kung, et al., 1981; Laroche and Bach, 1981; Haynes, et al., 1981; McMillan, et al., 1982c; Holden, et al., 1982a; Holden, et al., 1982b; Knowles and Halper, 1982; Haynes, et al., 1982a; Haynes, et al., 1982b; Schmitt, et al., 1982; Thomas, et al., 1982; Wood, et al., 1982; Thivolet, et al., 1982; Mackie and Turbitt, 1982; Hashimoto and Iwahara, 1983; Beuchner, et al., 1983; Sheibani, et al., 1983; Hofman, et al., 1983b; Willemze, et al., 1983a; Willemze, et al., 1983b; Chu, et al., 1984;

Abel, et al., 1985; Ralfkiaer, et al., 1985a; Ralfkiaer, et al., 1985b; Nasu, et al., 1985; Wood, et al., 1986) few have included simultaneous testing with B cell antibodies. Three studies which did, however, have also found B cell content to be insignificant (Sheibani, et al., 1983; Willemze, et al., 1983a; Nasu, et al., 1985). The results, therefore, contrast with those in pseudolymphomas (Chapter 7), and cutaneous lymphomas of non MF/SS origin (Chapter 6).

7. LeuM1/LeuM3; Esterase Positive Macrophages

The results obtained with the macrophage antibodies LeuM1/LeuM3 and esterase cytochemistry indicate further heterogeneity of CTCL infiltrates. Since LeuM1 reactivity may reside on some activated T cells it is possible that some of the LeuM1 reactive cells are lymphoid rather than histiocytic. However, no case with a LeuM1 preponderant phenotype is observed. The numbers of LeuM1+/LeuM3+ cells is similar to the estimate of macrophage content in CTCL using non-specific esterase (this chapter) or other macrophage McAbs (Wood, et al., 1982). Further information is required on functional aspects of LeuM1+/LeuM3+ cells before inferences can be made as to their possible roles in CTCL and benign lymphoid infiltrates. It is, therefore, likely that the situation contrasts with Hodgkin's disease where LeuM1 positivity is a conspicuous feature in Hodgkin/Reed Sternberg cells (Hsu and Jaffe, 1984c).

8. HNK1+ Lymphocytes

The results indicate that HNK1+ lymphocytes may be found in human lymphomatous lesions. It is uncertain whether this phenomenon is due to migration of the HNK1+ lymphocytes into the tumour or due to trapping of HNK1+ cells undergoing physiologic migration through the affected area. As mentioned later (Chapter 6) this may account in part for the presence of HNK1+ lymphocytes in one category of cutaneous lymphoma. The numbers of HNK1+ lymphocytes present are small, however, HNK1+ lymphocytes usually

forming <1% of the population in CTCL. It is unlikely that blood contamination of the frozen sections accounts for this positivity as examination of 30 sections from 5 scalp biopsies (healthy donors) (Chapter 4) did not reveal HNK1+ lymphocytes. Although it cannot be ruled out that the HNK1+ lymphocytes are tumour cells, this seems unlikely, as no tumour with a preponderant HNK1+ phenotype was observed. Since a subpopulation of HNK1+ cells are OKT3+ and most cases of CTCL have an OKT3+ phenotype, it could be argued that aberrant differentiation in the T cell tumour might result in a fraction of tumour cells with an OKT3+ HNK1+ phenotype. This question could be resolved by immuno-electron microscopy.

An HNK1+ phenotype does not necessarily imply that these cells are functioning optimally in terms of their migrational or cytolytic capabilities. Decreased NK function has previously been described in human lymphomas (Tursz, et al., 1982) including CTCL (Kragballe, et al., 1983).

The presence of HNK1+ lymphocytes in small numbers in benign dermatoses; eg., lichen planus 2 of 3 cases, ≤1% of cells (this chapter), allergic contact dermatitis 1 of 7 cases, <1% of cells (Chapter 4), indicates that HNK1+ lymphocytes are not restricted to malignant lymphoid populations. The small number of HNK1+ lymphocytes observed in these conditions makes it unlikely that they are the main instigators of the spongiotic and hydropic injury seen in these diseases. However, the finding of HNK1+ lymphocytes at the dermo-epidermal junction in lichen planus suggests that they may play a role in the formation of apoptosis and hydropic degeneration seen in this disorder. It is unknown whether the HNK1+ lymphocytes identified in 2 of 9 cases of LPAP (a potentially premalignant disorder) might be eliminating or controlling transformed lymphocytes.

The possibility that HNK1+ lymphocytes are present in

cutaneous lymphomas of non MF/SS type will be examined in the next chapter.

9. R423+ Dendritic Cells

Absence of R423+ dendritic cells contrasts with the consistent finding of OKT6+ dendritic cells in CTCL and benign T cell infiltrates. R423+ dendritic cells are, however, later demonstrated in cutaneous B cell lymphoma and pseudolymphoma (Chapters 6 and 7).

10. Be1/Be2.

The Be1/Be2 antigens are frequently present in cutaneous infiltrates of CTCL (Be1, 12/14 cases; Be2, 9/13 cases). The presence of Be1/Be2 positive cells in benign dermatoses such as lichen planus (Be1, 1/3 cases; Be2, 2/3 cases) and atopic dermatitis (Be1, 1/5 cases; Be2, 1/5 cases) (occasionally also in contact dermatitis, Chapter 4) indicates these reagents are not however specific for lymphoma when used on tissue sections. Reactivity of these reagents with further cutaneous disorders will be discussed in later chapters.

Salient features of recent studies of CTCL carried out by others using McAbs are outlined in Table 7, and are contrasted with the findings reported in this chapter (top line). There is considerable agreement between the results of various workers. The spectrum of reactivity of the immature markers OKT9, OKT10, and leukaemia-lymphoma antibodies Be1, Be2 with benign dermatoses, LPAP and CTCL is shown schematically in Figures 6 and 7.

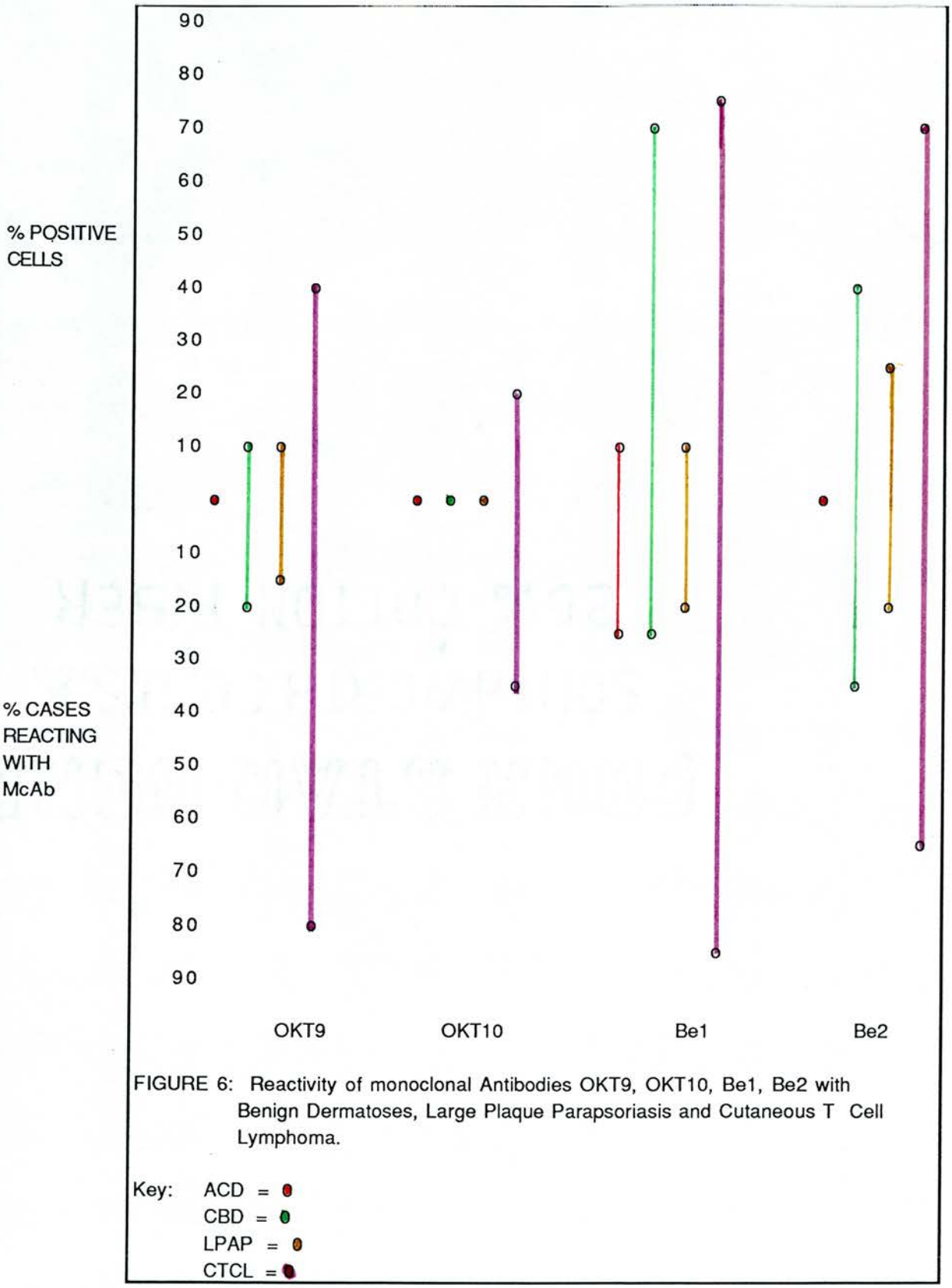
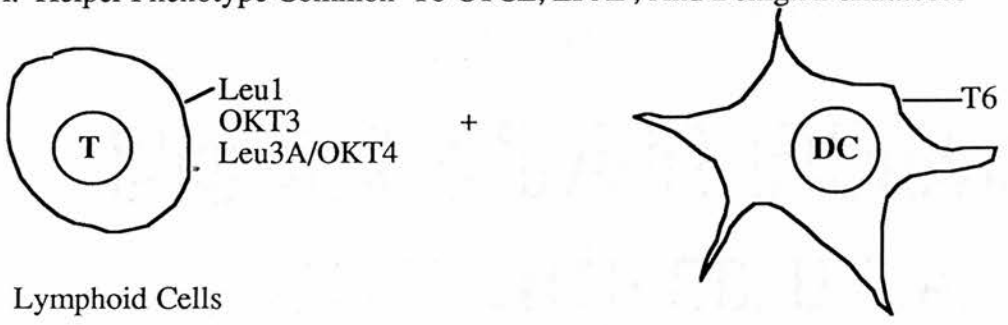


FIGURE 6: Reactivity of monoclonal Antibodies OKT9, OKT10, Be1, Be2 with Benign Dermatoses, Large Plaque Parapsoriasis and Cutaneous T Cell Lymphoma.

Key: ACD = ●
CBD = ●
LPAP = ●
CTCL = ●

A. Helper Phenotype Common To CTCL, LPAP, And Benign Dermatoses



Lymphoid Cells

Interspersed Dendritic Cells

B. Markers More Commonly Expressed In CTCL (Lymphoid Component)

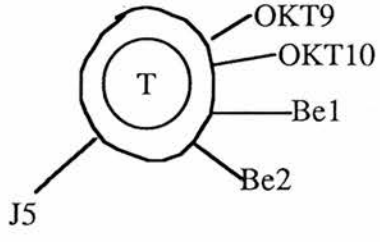


FIGURE 7: Schematic Representation of Comparitive Surface Antigen Expression of Cutaneous T Cell Lymphoma, Large Plaque Parapsoriasis, and Benign Dermatoses.

CHAPTER SIX

CUTANEOUS LYMPHOMAS OF NON-MYCOSIS/SEZARY TYPE

Including:

A. Introduction
B. Materials and Methods
C. Results
   Phenotype of Individual Cases Numbers 1-13
D. Discussion
   1. Lineage Assignment
      a) True Histiocytic Markers
      b) Dual Marker Expression
      c) Additional Histiocyte Markers (LeuM1, OKT6, Leu3A)
      d) Non-Specificity of HLADR, OKT9
      e) Requirement for Multiple McAbs
   2. Comparison with Normal T Cell Differentiation (Aberrant Phenotypes)
   3. OKT6 Reactivity
   4. Comparison with Other T Cell Lymphomas and Leukaemias
   5. E Receptor Antibody (T11)
   6. Leu1 specificity
   7. J5
   8. B Cell Markers
      a) Leu 14
      b) Kappa/Lambda Ratio and Concept of Monoclonality
      c) B1/B2 Expression and Comparison with Normal B Cell Differentiation
   9. Follicular Lymphoma
      Follicular Dendritic Reticulum Cells (DRC)
   10. Common Leukocyte Antigen 2D1
   11. Class I and II HLA Expression

12. Myeloid Antibodies
13. Macrophages (Esterase LeuM1, LeuM3)
14. Be1 and Be2
15. HNK1
16. Unclassifiable (U Cell) Types

CHAPTER SIX

CUTANEOUS LYMPHOMAS OF NON-MYCOSIS/SEZARY TYPE

A. INTRODUCTION

The involvement of skin by non-Hodgkin's lymphomas (NHL) of non MF/SS type has been described by several workers. (Bluefarb, 1959; Kim, et al., 1962; Ribero, 1972; Long, et al., 1976; Saxe, et al., 1977; Burke, et al., 1981)

The immunological typing of leukaemia lymphoma may have prognostic and therapeutic significance (Chapter 8).

Contrary to the tenets of the Lukes-Collins classification (Lukes and Collins, 1974) there is growing evidence that morphologic criteria alone are poor predictors of lineage. (Jaffe, et al., 1982; Bain, 1983) For instance, a) large cleaved follicle center B cell lymphomas may be confused with multilobated or convoluted T cell lymphomas; (Palutke, et al., 1980) b) small convoluted B cell lymphomas have recently been described and these can be confused with CTCL (Palutke, et al., 1980) c) Although morphologic criteria exist for differentiating B cell from T cell immunoblastic sarcomas (Levine, et al., 1981) the failure rate in such predictions is high.

Existing methods of lymphoma typing have resulted in a high proportion of unclassifiable cases because of expression of insufficient (Lukes, et al., 1978a and b) (NULL) or dual (Habeshaw and Stuart, 1975) markers. The ultimate role of immunologic characterization will, therefore, depend on more accurate methods of analysis.

Cutaneous lymphomas of non MF/SS type have been previously studied utilizing enzyme cytochemistry and heteroantisera although such studies are sparse. (Burg and Braun-Falco, 1978; Willemze, et al., 1982a; VanDerValk, et al., 1984b) A surprisingly high incidence of true histiocytic neoplasia has been reported by one group. (Willemze, et al., 1982a)

B. MATERIALS AND METHODS

The methods are as outlined in Chapter 3. Thirteen patients with cutaneous lymphoma are included in the study. These are classified (Table 8) as primary (P) if no evidence of concurrent disease was detected at the time of oncologic screening, concurrent (C) if cutaneous involvement was a presenting feature but systemic involvement was found on screening, and secondary (S) if cutaneous involvement developed subsequent to the diagnosis of NHL elsewhere. Lymphomas are classified according to the method of Rappaport. (Rappaport, 1966a) The histologic criteria for the diagnosis of cutaneous lymphoma have been described. (Evans, et al., 1979; Burke, et al., 1981) The panel of monoclonal antibodies used and their specificities are described under methods (Chapter 3). In addition to the macrophage/monocyte antibodies non specific esterase is used to detect histiocytes as this method has previously been found useful in determining the origin of "histiocytic" neoplasia (Burg and Braun-Falco, 1978; Willemze, et al., 1982a; VanDerValk, et al., 1984b). Because of loss of T cell markers in certain cases of CTCL (Chapter 6) a wide battery of reagents is used eg., 4 pan T cell markers (Leu1, OKT3, Leu4, T11). A leukocyte marker, (Pizzolo, et al., 1980) 2D1, is also employed to differentiate lymphoma from epithelial neoplasia and sarcomas in potentially "null" cases.

C. RESULTS

The results are summarized in Table 8.

With the monoclonal antibody panel used the 13 cutaneous lymphomas could be categorized into T cell (8 cases, Nos. 2,3,5,7,10,11,12,13) B cell (3 cases, Nos. 4,6,9) and unclassifiable or U cell (2 cases Nos. 1 and 8). When grouped by histologic type the distribution is as follows:

a) Diffuse Histiocytic: 8 cases, 4 T cell, 2 B cell,

TABLE 8-MONOCLONAL ANTIBODY TESTING OF CUTANEOUS LYMPHOMA OF NON-MF/SS TYPE

Patient	Histologic Diagnosis	Lineage Assignment	Leuk 2D1	Leu1	OKT3	MCT Leu 4 Leu5	Leu2A	Leu3A	OKT6	OKT9	OKT10	B1	B2	Leu14	K	L	J 5
1	DH (c)	U	++	-	+	-	-	++	-	++	Nt	-	-	-	-	-	Nt
2	DH (s)	T	++	++	++	Nt	-	++	+	++	-	-	-	-	Nt	Nt	-
3	DH (p)	T	++	+	Nt	++	+	+	+	++	-	-	-	Nt	Nt	Nt	-
4	DH (p)	B	++	+	+	Nt	+	-	-	-	Nt	++	++	Nt	Nt	Nt	Nt
5	DH (p)	T	++	++	-	Nt	+	++	+	++	+	-	-	Nt	Nt	Nt	-
6	DH (p)	B	++	+	+	+	+	+	-	-	Nt	++	-	++	++	+	Nt
7	DH (c)	T	++	++	++	Nt	-	++	-	++	Nt	-	-	Nt	Nt	Nt	Nt
8	DH (p)	U	++	+	+	Nt	+	++	-	+	-	-	-	Nt	Nt	Nt	-
9	NMLH (p)	B	++	+	+	+	+	+	-	+	+	++	+	++	++	+	-
10	LWD (p)	T	++	++	++	++	-	++	-	-	Nt	-	-	Nt	Nt	Nt	-
11	LWD (p)	T	++	++	Nt	Nt	-	+	+	+	-	-	-	Nt	Nt	Nt	Nt
12	LWD (p)	T	++	++	Nt	Nt	-	-	-	+	Nt	-	-	Nt	Nt	Nt	Nt
13	DMLH (LEG) (p)	T	++	-	Nt	++	+	++	++	++	Nt	-	-	-	-	-	-
	DH (lymph node)	T	++	-	Nt	++	-	-	-	++	Nt	-	-	-	-	-	Nt

Patient		NK1	NSE	DR	T11	M1	M3	R4	Be1	Be2	HLA	MY3	MY10	MY11	MY12	MY13
1	DH (c)	-	Nt	Nt	Nt	+	+	-	-	Nt	Nt	Nt	Nt	Nt	Nt	Nt
2	DH (s)	-	+	+	+	+	+	-	+	++	++	-	-	++	-	+
3	DH (p)	+	+	Nt	Nt	+	+	-	+	++	++	+	-	++	-	+
4	DH (p)	+	+	Nt	Nt	-	-	-	-	+	++	-	-	++	-	+
5	DH (p)	+	+	Nt	Nt	-	-	-	-	-	Nt	Nt	Nt	Nt	Nt	Nt
6	DH (p)	-	+	Nt	Nt	-	++	-	++	++	Nt	Nt	Nt	Nt	Nt	Nt
7	DH (c)	-	Nt	Nt	Nt	+	Nt	Nt	-	++	Nt	Nt	Nt	Nt	Nt	Nt
8	DH (p)	+	+	+	-	Nt	Nt	-	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt
9	NMLH (p)	+	+	+	+	+	+	++	+	++	++	+	-	++	-	-
10	LWD (p)	+	+	Nt	++	-	-	-	+	-	++	+	-	+	-	+
11	LWD (p)	Nt	Nt	Nt	Nt	Nt	Nt	Nt	+	-	Nt	Nt	Nt	Nt	Nt	Nt
12	LWD (p)	-	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt
13	DMLH (LEG) (p)	Nt	Nt	+	++	+	Nt	Nt	-	-	-	Nt	Nt	Nt	Nt	Nt
	DH (lymph node)	-	-	+	++	Nt	+	-	-	-	-	Nt	Nt	Nt	Nt	Nt

KEY: DH: Diffuse histiocytic
NMLH: Nodular mixed lymphocytic histiocytic
LWD: Diffuse lymphocytic well differentiated
DMLH: Diffuse mixed lymphocytic histiocytic
MCT: Multiclone mixture of Leu4 and Leu5
Nt = Not tested, - = Negative Reaction,
+ = ≤ 50% of cells staining, ++ > 50% of cells staining

C: Concurrent, ie., cutaneous involvement presenting feature but systemic involvement found on screening
S: Secondary, Ie., cutaneous involvement developed subsequent to the diagnosis of NHL elsewhere
P: Primary, ie., no evidence of concurrent disease detected at the time of oncologic screening

2 U Cell.

b) Nodular Mixed Lymphocytic Histiocytic: 1 case, 1 B cell.

c) Diffuse Lymphocytic Well Differentiated: 3 cases, 3 T cell.

d) Diffuse Mixed Lymphocytic Histiocytic: 1 case, 1 T cell.

e) Diffuse Histiocytic (Nodal): 1 case, 1 T cell.

Testing with 2D1 (13 cases) and HLA (5 cases) is uniformly positive.

Phenotype of Individual Cases

The rationale behind assignment of phenotype to individual cases will now be described.

Case 1. The negative reaction with pan T (Leu1, OKT3, Leu4/5) and B markers (B1, B2, Leu14, Kappa, Lambda) makes this case unclassifiable (U type). A positive reaction with Leu3A and OKT9 (Plate 44) would be compatible with a macrophage tumour or T cell tumour showing aberrant differentiation. Support for the former possibility could not be obtained with LeuM1/M3 which stain a minority of infiltrating cells. Weak staining of tumour cells with mature T cell marker OKT3 suggests this tumour is probably T cell derived with loss of mature surface antigens.

Case 2. (Plates 45, 46, 47) Reactivity of the majority of tumour cells with pan T (Leu1, OKT3) and negative reaction with B markers permits designation as a T cell neoplasm. Leu3A and 3A1 positivity (Plate 47) in the presence of a Leu1+, OKT3+ reaction indicates a helper T cell phenotype. Despite the positive reaction in the majority of cells with Leu1, and OKT3, a minority of cells stain with E receptor antibody T11 suggesting aberrant differentiation.

Case 3. T cell identity is indicated by reactivity with Leu4/5 and negative reaction with B markers. However, Leu1 (pan T) stains a minority of cells (aberrant differentiation).

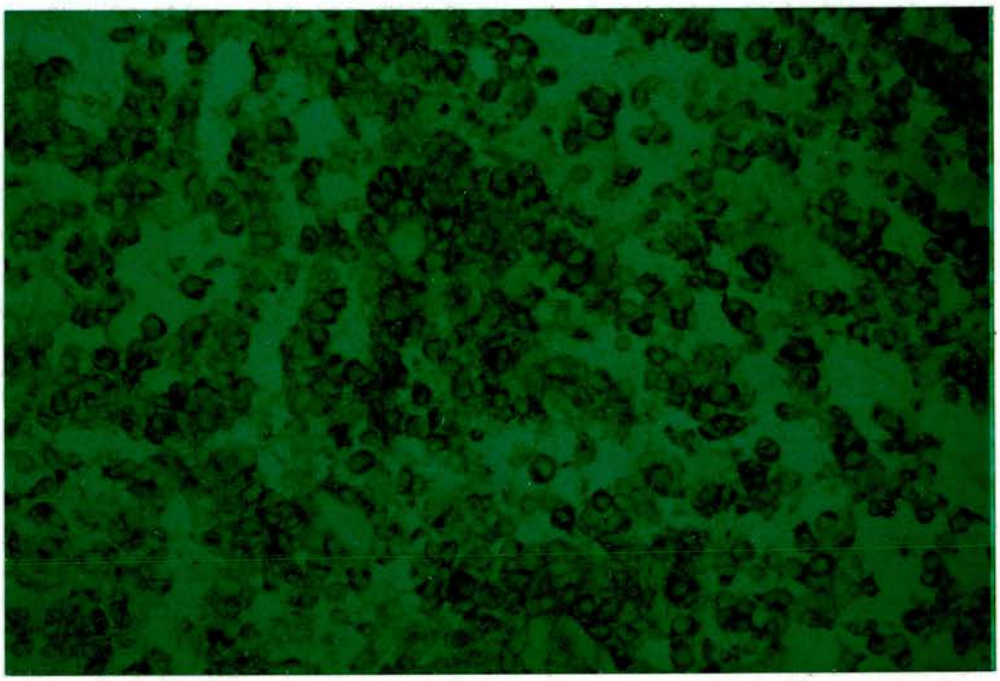


PLATE 44. **LARGE CELL LYMPHOMA.** (Phenotyically unclassifiable) OKT9+ cells. Weak staining with OKT3 suggests this tumour is T cell derived. Indirect immunoperoxidase. Non counterstained. Green filter x 64.

PLATE 45. **LARGE CELL LYMPHOMA.** T cell type. Cutaneous ulcerated plaques on back, right flank and right axilla.

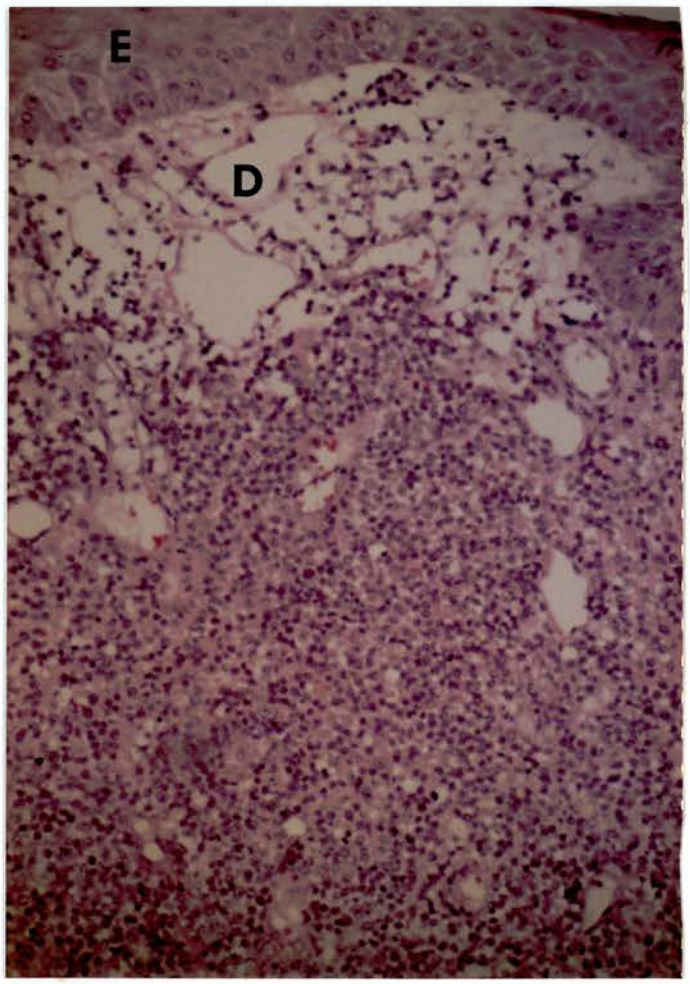


PLATE 46. **LARGE CELL LYMPHOMA.** T cell type. Predominantly non epidermotropic infiltrate. Epidermis (E). Dermis (D). Haematoxylin and Eosin x 40.

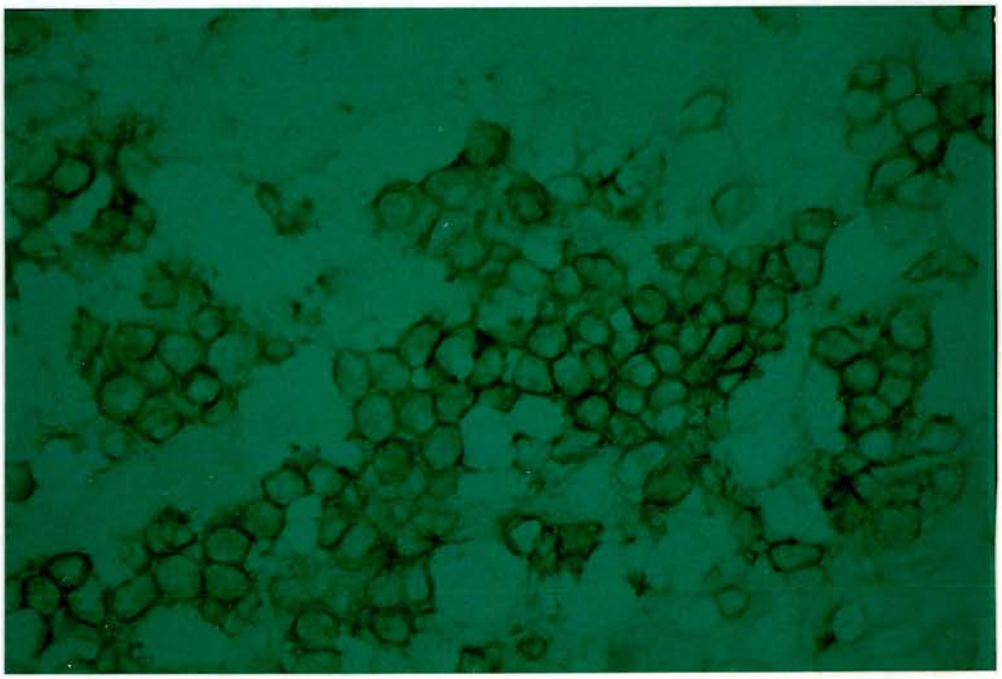


PLATE 47. **LARGE CELL LYMPHOMA.** T cell type. 3A1+ (T cell, T subset) dermal tumour cells. Similar staining observed with Leu1 (mature T) and Leu3A (helper T) but not with T11 (E receptor), indicating aberrant differentiation. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

Case 4. This tumour is assigned a B cell phenotype on the basis of reactivity with B1 and B2. A minority of small lymphocytes infiltrating the tumour are Leu1+, OKT3+, Leu2A+ suppressor T cells.

Case 5. The Leu1+, OKT3-, B1-, B2- phenotype implies a T cell tumour with associated loss of the OKT3 marker (aberrant differentiation).

Case 6. B1, Leu14 (pan B) positivity in the majority of cells and reactivity in a minority of cells with Pan T McAbs (Leu1, OKT3) indicates a B cell identity. Testing with Kappa/Lambda reagents shows an almost pure population of Kappa+ cells. (monoclonality).

Case 7. A Leu1+, OKT3+, Leu3A+, B1-, B2- helper T cell phenotype is present.

Case 8. A Leu3A+ phenotype was present as in case 1 with similar possibilities of T cell tumour with aberrant differentiation or macrophage (true histiocytic) neoplasm. The latter possibility cannot be established with non-specific esterase as a minority of cells show diffuse cytoplasmic positive staining. The presence of Leu1, OKT3 positivity in a component of infiltrating cells suggests the possibility of varying degrees of differentiation within a thymic derived (T cell) neoplasm.

Case 9. The majority of large cells within the lymphoid follicles of this nodular lymphoma are B cells (Plate 48) (B1+, Leu14+, HLADR+) with a minority of admixed B2+ cells. B2 shows both rounded and dendritic staining pattern. R423+ dendritic cells are concentrated within lymphoid follicles (Plate 49). Leu1+, OKT3+, Leu4/5+, T11+ T cells are present within and between adjacent LFs with Leu3A+ (helper) outnumbering Leu2A+ (suppressor) cells in both locations. Where mantle zone architecture is retained the majority of lymphocytes are Kappa+ B cells (Kappa:Lambda, 2:1) with an admixture of Leu1+, OKT3+, Leu4/5+, T11+ T cells. However, within the neoplastic germinal centre and within

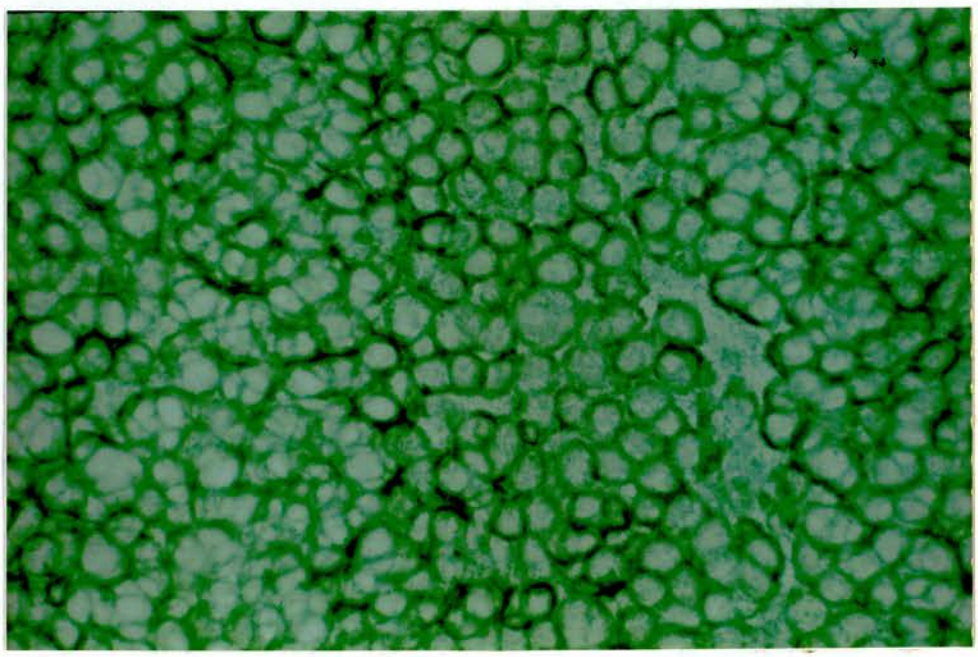


PLATE 48. **NODULAR LYMPHOMA.** Follicular Leu14+ B cells. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

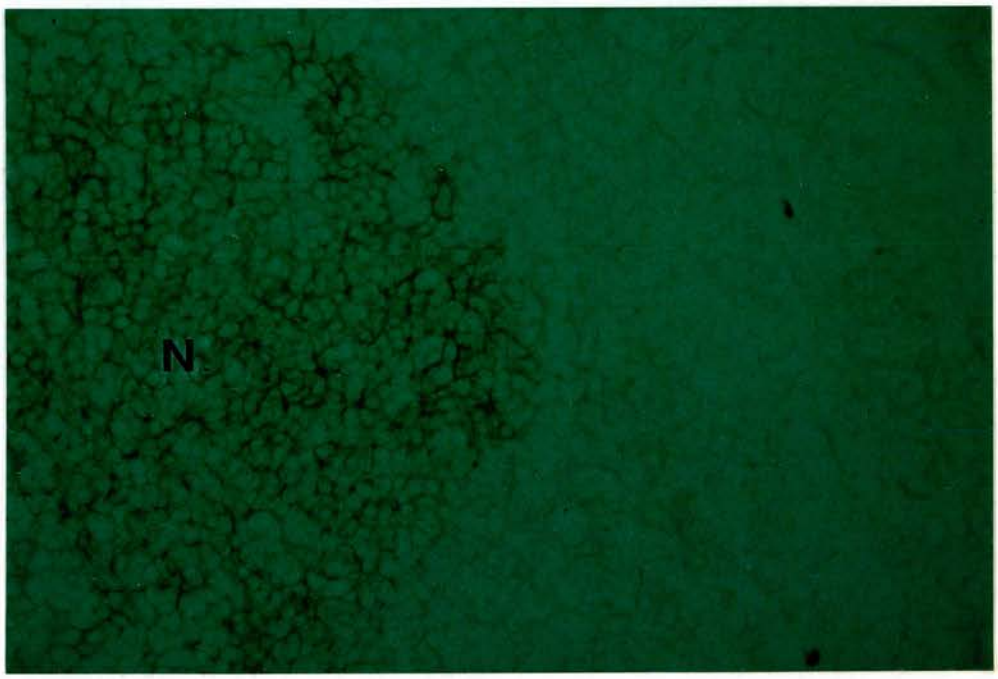


PLATE 49A. **NODULAR LYMPHOMA.** Tumour nodule (N) contains large number of R423+ follicular dendritic cells, similar to lymphoid follicles of non-malignant lymphoid tissue (tonsil). Indirect immunoperoxidase. Non counterstained. Green filter x 64.

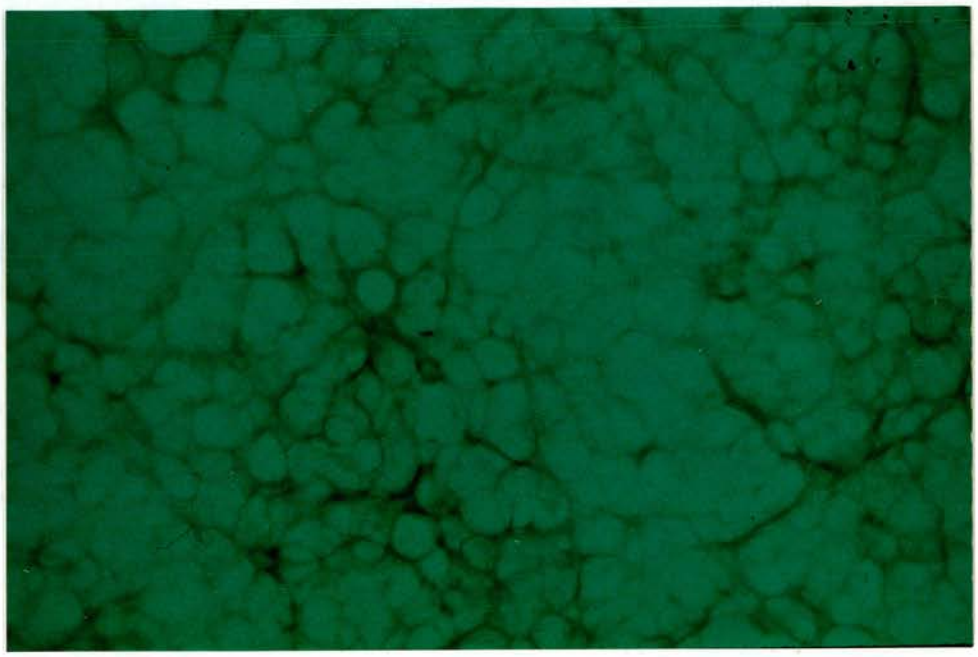


PLATE 49B. **NODULAR LYMPHOMA.** R423+ follicular dendritic cells interspersed throughout tumour nodules. Indirect immunoperoxidase. Non counterstained x 100.

nodules where good mantle zone preservation is not evident the Kappa/Lambda ratio is higher (4:1). In some areas the tumour nodule reaction is weak or negative with Kappa and Lambda reagents. Esterase positive macrophages are frequent within tumour nodules and occasionally found between them. The macrophage antibodies produce contrasting staining with LeuM3+, My3+ cells virtually confined to lymphoid follicles and LeuM1+ cells (Plate 50) present solely within the interfollicular areas.

A weak staining pattern of the tumour nodules is frequently observed with Leu1. This can, however, be easily discerned from the intense Leu1+ staining found in infiltrating small T lymphocytes.

A hazy equivocal or negative staining pattern is obtained with J5.

HNK1+ lymphocytes (Plates 51, 52) tend to be most evident between tumour nodules in contrast to their intrafollicular location in non-malignant lymphoid tissue (Chapter 4).

Reactivity with anti-transferrin receptor antibody OKT9 is often evident within the neoplastic nodules.

Cases 10, 11, 12. On the basis of reactivity of the majority of infiltrating cells with T cell McAbs and negative reaction with B cell McAbs these three diffuse well differentiated lymphocytic proliferation are assigned a T cell phenotype.

Case 13. As discussed in Chapter 8, the cutaneous histology of this case is malignant from a morphologic standpoint. The clinical course and microscopy are consistent with lymphomatoid papulosis type A. The cutaneous biopsies of this case were labelled by Doctor Costan Berard as cutaneous lymphoma mixed lymphocytic-histiocytic type prior to the development of nodal lymphoma. Irrespective of the microscopic semantics involved the phenotype of the cutaneous infiltrates is that of a Leu1-, Leu4/5+,

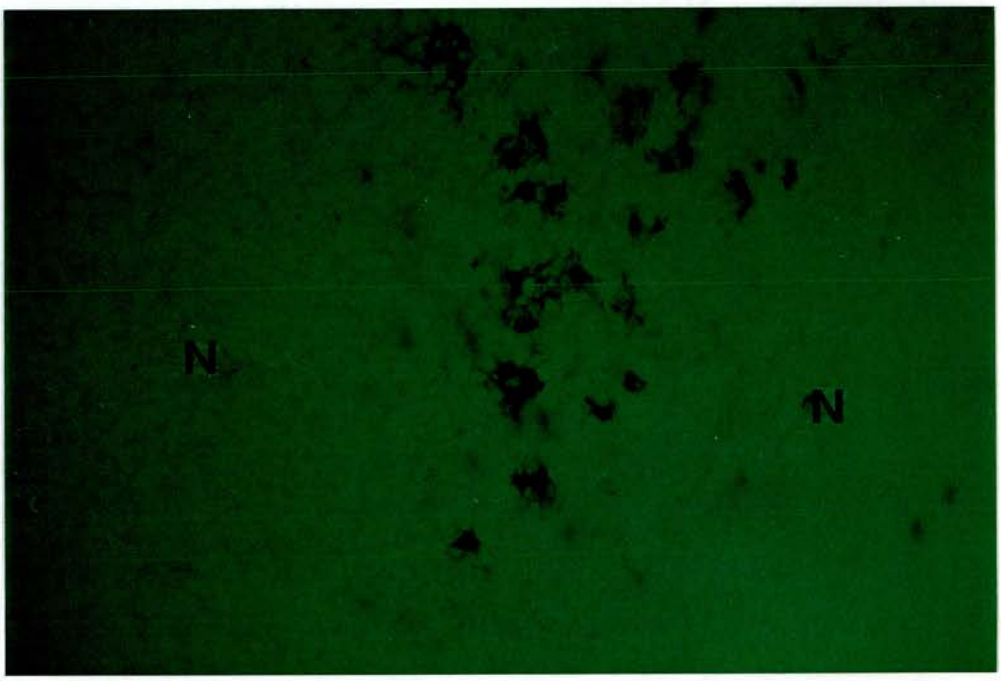


PLATE 50. **NODULAR LYMPHOMA.** LeuM1+ macrophages located between tumour nodules (N) in a manner reminiscent of that found in non-malignant lymphoid tissue (tonsil). Indirect immunoperoxidase. Non counterstained. Green filter x 40.

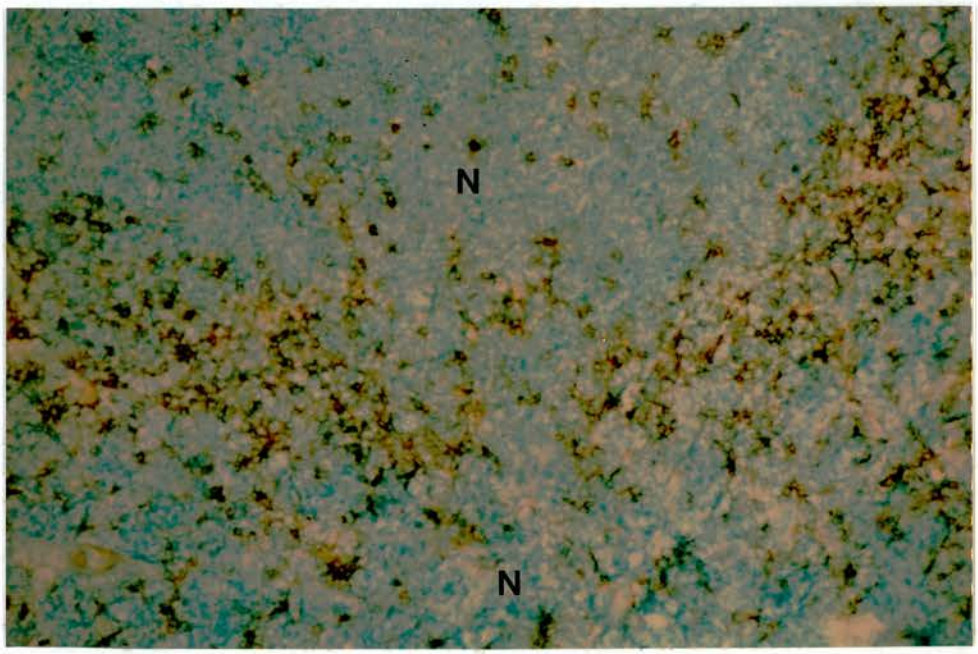


PLATE 51. **NODULAR LYMPHOMA.** Band of HNK1+ lymphocytes between tumour nodules (N) contrasts with normal follicular distribution of HNK1+ lymphoid cells in non-malignant lymphoid tissue. (C.f. Plate 10). Indirect immunoperoxidase. Haematoxylin counterstain x 40.

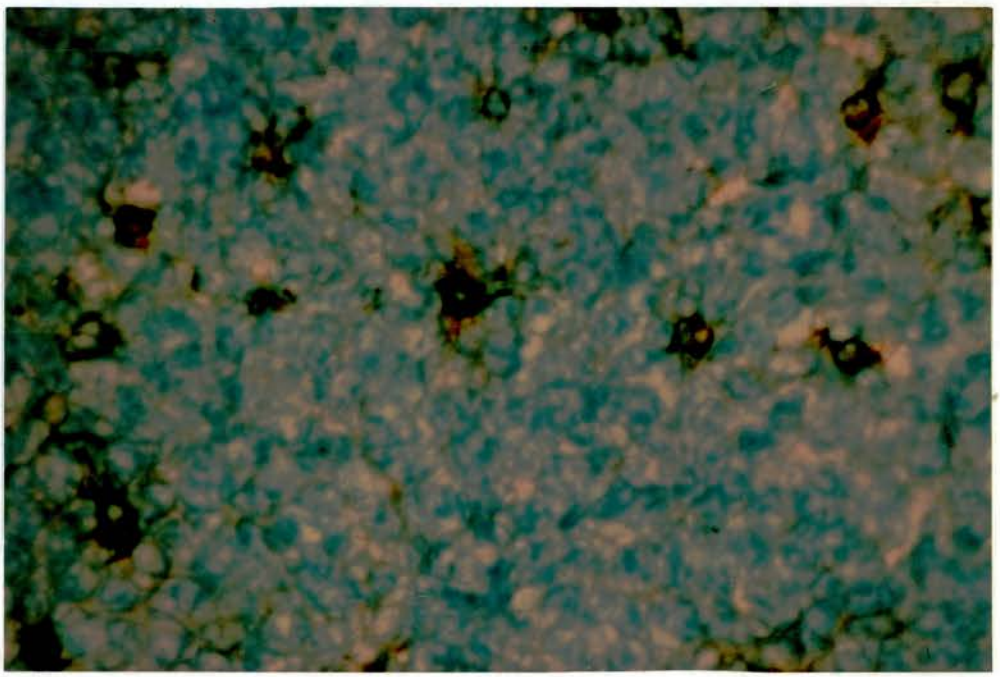


PLATE 52. **NODULAR LYMPHOMA.** High power view of HNK1+ lymphoid cels in dermal infiltrate. Indirect Immunoperoxidase. Haematoxylin counterstain x 160.

Leu3A+, HLADR+ helper T cell expressing aberrant differentiation (Leu1-) and the transferrin receptor (OKT9+). The phenotype of the large atypical histiocytic Reed-Sternberg like cells has been described in Chapter 7. The nodal infiltrate (more fully described in Chapter 7) can only be assigned T cell status after testing with Leu4/5. Again the differentiation status is aberrant with lack of expression of Leu1 (pan T) and subset markers (Leu2A/Leu3A).

D. DISCUSSION

1) Lineage Assignment

The majority of lymphomas studied (8/13) are of the diffuse histiocytic (Rappaport)/large cell (International Working Formulation, 1982) category.

A preponderance of large cell types (44%) in cutaneous lymphoma has previously been noted (Burke, et al., 1981) Ultrastructural studies of lymphocyte transformation indicate that lymphocytes may acquire morphological characteristics similar to histiocytes. (Andre-Schwartz, 1964; Wiener, et al., 1967) The "histiocytic" cases in this study are characterized into T (50%), B (25%) and U Cell (25%).

a) True Histiocytic Markers

Although B cell tumours form 3/13 lymphomas studied (2 histiocytic/LCL, 1 nodular mixed lymphocytic histiocytic) no tumours consisting predominantly of the other two components of the follicular compartment (macrophages and follicular dendritic cells, Chapter 4) are identified by the macrophage/D cell markers, NSE, LeuM1/M3, or R423. The components of nodular MLH lymphoma will be discussed later.

The results, therefore, contrast with those of Willemze et al. who found 66% of cutaneous large cell lymphomas to be true histiocytic, when using enzyme cytochemistry (NSE) and heteroantisera to lysozyme as histiocytic markers. (Willemze, et al., 1982a) An independent study by Wood et al. using McAbs directed against T cells, B cells, and

macrophages produced similar results to those outlined here with LCL comprising 71% of cutaneous lymphomas, 47% of these being T cell, 40% B cell and 13% U cell. (Wood, et al., 1983b).

Investigations of nodal LCL have generally shown a preponderance of B cell with few T cell or true histiocytic types. (Warnke, et al., 1980; Knowles, et al., 1982) It may be argued that the prevalence in this study of T cell tumours compared with previous studies of nodal lymphoma may reflect a generalized affinity of T cells for the skin.

b) Dual Marker Expression

No cases exhibit dual expression of T and B markers, a phenomenon previously demonstrated in leukemia lymphoma with a different series of surface markers. (Chapel and Ling, 1977)

c) Additional Histiocyte markers (LeuM1; OKT6, Leu3A)

Although LeuM1 reactivity may be found on activated T cells (Hanjan, et al., 1982) no cases are found here with expansion of a LeuM1+ neoplastic population as may be found in Hodgkin's disease where the R-S cells are LeuM1+, OKT9+. (Straughen and Dimitriu-Bona, 1986) (Hsu, et al., 1986) Likewise no cases demonstrate the OKT6+, HLADR+, Leu3A+ phenotype characteristic of Histiocytosis X (Chapter 7).

A similar lack of a LeuM1+ phenotype in nodal LCL and peripheral T cell lymphoma has been described by Hsu et al. (Hsu and Jaffe, 1984c)

d) Non-Specificity of HLADR, OKT9

An HLADR+ (Ia+) phenotype is present in 4:4 specimens tested. The HLADR antigens are useful in determining the B or T cell origin of normal peripheral blood lymphocytes as the majority of peripheral blood and tonsillar B cells (Chapter 4) express HLADR. (Winchester, et al., 1976)

Since HLADR antigens are found on only a small proportion of human peripheral blood T cells (Fu, et al., 1978) and certain activated T cells, (Evans, et al., 1978)

(unlike the mouse where Ia+ T cell subsets have been well characterized), it has been suggested that HLADR antigens may form a useful marker of B cell derived tumours. (Halper, et al., 1980) The finding of HLADR positivity in 2 T cell lymphomas (cases 2 and 13) and the frequency of T cell tumours in cutaneous lymphoma of non MF/SS type suggests HLADR alone will be of limited use in establishing neoplastic lineage.

Likewise, although the OKT9 antigen was originally described on immature T cells and thymocytes it is found here to be present on T and B cell tumours. Knowles, et al. (Knowles, et al., 1982) also found OKT9 to be of minimal value in assigning lineage.

The non-specificity of HLADR and OKT9 in lineage assignment is compatible with the findings in non-malignant lymphoid tissue in Chapter 4.

e) Requirement for Multiple McAbs

The combination of T cell and B cell markers permits the identification of T cell or B cell lymphoma in 11 of 13 cases tested. Difficulties caused by overlap of monoclonal markers, as may occur in CLL, (Royston, et al., 1980) were not encountered. However, these experiments establish a clear requirement for multiple T cell monoclonals when attempting accurate lineage analysis. For instance, cases 3 and 13 were considered U cell prior to the availability of the T cell markers, Leu4/5.

2) Comparison with Normal T Cell Differentiation (Aberrant Phenotypes)

Seven of eight cases of T cell lymphoma show phenotypes not found on normal mature peripheral T cells (Table 9).

Some of the differences observed (eg. OKT9, HLADR expression) may be representative of cellular activation (analogous to stimulation of normal lymphocytes by antigen or phytohemagglutinin (PHA)) or may be a function of active proliferation (eg. OKT9+). Loss of markers could represent

TABLE 9

ABERRANT PHENOTYPES IN CUTANEOUS NON-HODGKIN'S LYMPHOMA OF NON-MYCOSIS SEZARY TYPE

	Phenotypic Loss*	Phenotypic Gain
2	T11	OKT9 HLADR
3	Leu1	OKT9
5	OKT3	OKT9
7	- - -	OKT9
10	Leu4/5	- - -
12	Leu2A/Leu3A	
13 (Skin)	Leu1	OKT9 HLADR
Lymph Node	Leu1 Leu2A/Leu3A	OKT9 HLADR

*Since each case was not tested with the full range of markers these cases probably represent an underestimate of the range of phenotypic abnormalities in this group of patients with cutaneous lymphoma.

immaturity (reversal of or lack of differentiation) or actual aberrant differentiation with the presence of phenotypes not known to occur during normal thymic differentiation. For instance, case 2 (OKT3+, Leu1+, Leu3A+, OKT9+) does not fit with any known category of intrathymic differentiation (Reinherz and Schlossman, 1980) but may be similar to that of the physiologically transformed helper T cell in vitro. (Kung, et al., 1980) However, an OKT3+, Leu1+, Leu3A+, OKT9+ phenotype is not common in acute allergic contact dermatitis (Chapter 4). If cases one and eight are also T cell then these also represent unusual phenotypes. Case 11 (Leu1+, Leu2A-, Leu3A-) does not fit with any presently known category of physiological lymphocyte transformation or intrathymic differentiation. Knowles at al. (Knowles, et al., 1982) in concurrent studies using OKT subset McAbs have described similar phenotypes in systemic NHL eg. Leu1+, OKT8-, OKT4- (similar to case 11: Leu1+, Leu2A-, Leu3A-); Leu1-, OKT3-, OKT8-, OKT4+ (similar to cases 1 and 8). Both categories in their study expressed strong helper function in vitro confirming their T cell nature. Bach et al. (Bach, et al., 1981) described two cases with phenotypes similar to case 11 (OKT4-), which were functionally helper T. Interestingly enough McAbs directed at non-competing epitopes of OKT4 (OKT4A, OKT4B, OKT4C, OKT4D, OKT4E) did react with the OKT4- cells.

Wood, et al., (Wood, et al., 1983b) using the Leu series of reagents found two cases with phenotypes similar to cases 1 and 8 (Leu1-, Leu2A-, Leu3A+) and categorized these as T cell despite negative reactions in the majority of cells with a battery of pan T reagents. Their approach to lineage assignment was, therefore, more liberal than the one here but their overall results showed a similar requirement for multiple reagents in categorizing a particular lymphoma.

3) OKT6 Reactivity

None of the cases studied showed cerebriform morphology as in the case described by Kim and Winkelmann. (Kim, et al., 1962) Despite this, interspersed OKT6+ dendritic cells are observed in 5 of 8 T cell lymphomas in a manner similar to that found in MF and SS (Chapter 5). No case expresses a preponderantly OKT6+ phenotype as may be seen in cutaneous multilobated T cell lymphoma (VanDerPutte, et al., 1982a) or Histiocytosis X (Chapter 7). The 3 B cell and 2 U cell lymphomas are notably devoid of an OKT6 reactive component.

4) Comparison with Other T Cell Lymphomas and Leukaemias

The results of this study of cutaneous lymphoma of non MF/SS type, therefore, contrast with those in acute T cell ALL, (Reinherz, et al., 1980) lymphoblastic lymphoma, (Bernard, et al., 1981) and MF/SS (Chapter 5) where the majority of cases can be classified respectively into early, common and late thymocyte types.

As in MF/SS (Chapter 5) no cases with a Leu2A+ suppressor phenotype are identified. In contrast Leu3A+ preponderance is observed in 5 of the 8 T cell lymphomas. A majority of Leu3A+ cases was also noted by Wood et al. (Wood, et al., 1983b) and in this respect the NHL's are similar to MF/SS (Chapter 5). Only one case (#10) , however, has an overall phenotype (Leu1+, Leu3A+) identical to the majority of MF/SS, and in this case OKT6+ dermal cells are totally absent unlike the situation normally found in the latter group of conditions.

5) E Receptor Antibody (T11)

Loss of T11 (E receptor antibody) in >50% of cells with retention of OKT3, as in case 2, is reminiscent of the E receptor negative OKT3+ cases of T cell ALL reported by Sondel (Sondel, et al., 1981). However, E rosetting capacity and T11 reactivity cannot be equated since cases of lymphoma have been described which fail to form E rosettes (E-) but react with T11 (T11+) (Knowles, et al., 1982).

One similar case which was Leu1+, Leu4+ (OKT3+) Leu5- (T11-) occurred in Wood's series (Wood, et al., 1983). The latter study utilized a very similar battery of McAbs to the ones in this study but exact comparisons are difficult since the Stanford group did not simultaneously utilize any potential markers of immaturity such as OKT9.

6) Leu1 Specificity

The presence of Leu1 (OKT1) reactivity in CLL (Royston, et al., 1980) (generally thought to be a B cell neoplasm in view of its E- SIg+, B1+ phenotype) and low grade NHL's (ie., small lymphocytic, mantle zone, some follicle centre cell derived lymphomas) (Knowles, et al., 1982; Cossman, et al., 1984) suggests Leu1/T101/OKT1 should be used alone with caution as markers of T cell lymphoma without simultaneous use of pan B reagents such as B1. Of the three B cell lymphomas in this particular study, (cases 4, 6, and 9) only case 9 (nodular mixed lymphocytic histiocytic) shows weak Leu1 expression of some tumour cells. This was easily differentiated from the intense staining observed in infiltrating T cells.

7) J5

Of the seven cases tested with J5 (5T, 1B, 1U) no positively staining cases are observed. This antigen which is present on some foetal and newborn thymocytes and occasionally in CTCL (Chapter 5), may be found on cutaneous lymphoblastic lymphomas of pre B type (Bernard, et al., 1982). Testing of further cases will be required to determine its true prevalence in cutaneous lymphoma.

Although it may be expressed by the neoplastic cells of follicular centre B cell lymphomas (Ritz, et al., 1981) the reaction with J5 in case 9 is negative, as in non-neoplastic lymphoid follicles (Chapter 4). However, as discussed in Chapter 4, highly sensitive techniques such as the avidin-biotin peroxidase method may pick up low levels of expression of this antigen not detected by the indirect

immunoperoxidase technique.

8) B Cell Markers

B cell lymphomas are detected here by their general reactivity with McAbs Leu14, B1, B2, anti-Kappa/Lambda and general lack of reactivity with T cell McAbs.

a) Leu14

Leu14 was provided prior to its commercial release so that data on lymphoma reactivity with this antibody are sparse. Leu14 reacts with B cells and has weak reactivity with monocytes but not macrophages. Leu14 is positive in one nodular mixed lymphocytic histiocytic (B1+) lymphoma and one diffuse large cell (B1+) lymphoma. The remaining cutaneous B cell lymphoma (CBCL) case was not tested. A negative reaction is obtained in the two T cell and one Leu3A+ U cell lymphoma examined.

Positive reactions have also been obtained with this marker in pseudo-lymphomas of B cell type (Spiegler Fendt Sarcoid) but not in lymphomatoid papulosis, a T cell pseudo-lymphoma (Chapter 7).

b) Kappa/Lambda Ratio and Difficulties in Implementing the Concept of Monoclonality

The anti-Kappa/Lambda reagents show a Kappa/Lambda ratio of >5:1 in case 6 (diffuse large cell) and 4:1 in case 9 (nodular mixed lymphocytic histiocytic). The Kappa/Lambda ratio in the latter is lowered to some extent by the presence of polyclonal mantle populations around some lymphoid follicles where the K/L ratio approaches the more normal ratio of 2:1.

Studies of lymphoma clonality are based on the fact that: 1) Individual lymphoid cells are restricted in their potential for immunoglobulin (Ig) expression and that these restrictions are maintained within their progeny (Nisonoff, et al., 1975). 2) A malignant tumour is thought to represent the proliferation of a clone of cells arising from a single transformed cell (Friedman and Fialkow, 1976).

Evidence for this concept has come from karyotype analysis (McMillan, 1973; Moore, et al., 1974) isoenzyme patterns (Lyon, 1972), and immunoglobulin expression (Preud'Homme and Seligmann, 1972).

Monoclonality of B cell neoplasia may be inferred from their uniform restriction to a single light chain type (Gearhart, et al., 1985) and the demonstration that heavy chain production is not restricted clonally (Levy, et al., 1977) has resulted in light chain ratios being used as the major criterion for monoclonality.

The actual criteria used for monoclonality vary. Some investigators insist that the tumour population must stain exclusively for one light and or heavy chain (Sarage, et al., 1981), some insist on a K/L ratio of >5:1; (Starzl et al., 1984) whereas, others regard a K/L ratio of $\geq$3:1; (Levy, et al., 1983) as "monoclonal". By the first set of criteria neither case 6 nor case 9 would have been monoclonal, by the second set case 6 would be monoclonal, and by the third set both would be monoclonal.

Case 9 exemplifies 2 technical difficulties which can obscure a monoclonal pattern, namely the presence of presumptive non-neoplastic polyclonal lymphocytes (mantle zone in this particular example) and non-marking neoplastic lymphocytes which can dilute the neoplastic SIg bearing population. The former has also been noted by Harris et al. (Harris, et al., 1984) and the latter by Warnke et al. (Warnke and Levy, 1978). In fact Taylor (Taylor, 1976) reported that few if any follicular-lymphoma cells stained for Kappa or Lambda. This latter finding may, however, have been complicated by alterations of Ig from fixation (Warnke, et al., 1978).

It should also be emphasized that monoclonality does not equate with the seemingly autonomous proliferation usually associated with malignancy as lymphomas showing monoclonal patterns in transplant patients have reversed on

discontinuing immunosuppression (Starzl, et al., 1984) and monoclonal K/L ratios have been described in apparently benign lymphoid hyperplasia (Levy, et al., 1983) (although follow-up was limited to less than 1 year in many cases).

The application of K/L markers to cutaneous lymphocytic infiltrates has been infrequent. The reagents used in this study are among the first anti-K/L McAbs produced. The results obtained in cases 6 and 9 indicate they will be useful in future studies of CBCL.

c) B1/B2 Expression and Comparison With Normal B Cell Differentiation

Anti-Kappa/Lambda reagents probably cannot be used alone for the identification of CBCL since a proportion of NHL are Ig negative (Froland and Natvig, 1970). In this respect the results with B1 and B2 are of interest.

Human B cells have previously been identified by heavy chain isotypes of immunoglobulin (Froland and Natvig, 1970), HLAD related Ia-like antigens (Humphreys, et al., 1975), receptors for Fc portion of Ig G (Huber, et al., 1969), receptors for the C3 component of complement (Bianco, et al., 1970), and for murine and monkey erythrocytes (Pellegrino, et al., 1975).

Many of these markers are limited by their lack of specificity for B lymphocytes. For instance, complement receptors do not always distinguish between T and B lymphocytes since normal T cells (Bankhurst, et al., 1978) activated T cells and occasional cases of T cell leukemia (Toben and Smith, 1977) and lymphoma (Pinkus, et al., 1979) may have them.

B1/B2 are distinct from previously described B cell surface markers including SIg, Ia-like antigens, Fc and C3 receptors. B1 (Stashenko, et al., 1980; Nadler, et al., 1981a) is present on >95% of B cells in peripheral blood and lymphoid organs. B1 is expressed on virtually all tumours of B cell origin with the exception of myeloma. (Nadler, et

al., 1981a) It is also present on a proportion of non-T, non-B acute lymphatic leukemia of "pre-B" type (Nadler, et al., 1981a). B2 has a similar cellular distribution but in contrast to B1 is only weakly expressed on peripheral blood B cells (Nadler, et al., 1981b). B2 shows more heterogeneous expression on B cell malignancies, being present on 90% of CLL, 50% of diffuse and nodular poorly differentiated lymphomas, and 10% of acute lymphatic leukemia cells but is absent in Burkitt lymphoma, Waldenstrom macroglobulinemia, and myeloma (Anderson, 1984).

B1 spans most stages of B cell differentiation being detected before the development of cytoplasmic mu-chains (mu+ pre-B cells) and is lost at the secretory stage of B cell differentiation (Stashenko, et al., 1981). The B2 antigen expresses distinct binding sites for C3d component of complement (Ida, et al., 1983). B2 appears on the cell surface after the cytoplasmic mu pre-B stage (Nadler, et al., 1984), and is lost earlier than B1 at a time when surface IgD is no longer detectable (Stashenko, et al., 1981). A small population of B cells stains with B2 and lacks B1 (B1-, B2+) (Anderson, et al., 1985). During B cell activation phenotypic alterations of the B cell surface occur including loss of B2, B1 and IgD with acquisition of SIgG and T10 (Stashenko, et al., 1981).

Tumours which are thought to correspond to an "early" stage of B cell differentiation, eg., B CLL, are B1+ B2+ whereas those corresponding to later stages of differentiation or transformed B cells (Waldenstrom's macroglobulinemia, plasma cell myeloma, large cell lymphomas) are B1+ B2- (Nadler, et al., 1981b).

A hypothetical model of normal B cell differentiation and that of the malignant B cell, adapted from a recent publication (Anderson, et al., 1984), is shown in Table 10. Using these criteria Case 6 (diffuse histiocytic/large cell; B1+ B2-) appears to correspond to a later stage of B cell

TABLE 10- HYPOTHETICAL MODEL OF B CELL DIFFERENTIATION RELATING MALIGNANT B CELLS AND THEIR NORMAL COUNTERPARTS

ANTIGEN			NON-T	ALL		CLL	DIFFUSE	NODULAR	LARGE CELL	WALDENSTROM	MYELOMA
		10%	30%	40%	20%		PDL	PDL	D OR N		
Ia	+	+	+	+	+	+	+	+	+	+	±
B4		+	+	+	+	+	+	+	+	±	
CALLA			+	+	+			+	±		
B1				+	+	+	+	+	+	+	
Cyto mu					+						
B2						+	+	+			
T1(Leu1)						+					
SIgM						+	±	±	±		
SIgD						+					
SIgG							±	±	±	±	
CytoIgG										+	+
T10										+	+
PCA1										+	+
PC1										±	+
II	B CELL PROGENITOR		PRE-B	CELL		IMMATURE B		VIRGIN B	LYMPHOBLAST	PLASMABLAST	PLASMA CELL

Key: + Positive staining
± Variable staining

differentiation than Case 4 (diffuse histiocytic/large cell; B1+ B2+) and Case 9 (nodular mixed lymphocytic histiocytic; B1+ B2+-). Case 4 is unusual in its B2+ phenotype as diffuse LCLs, which are thought to correspond to the transformed B cell are generally B2- (Anderson, et al., 1985). However, occasional cases are B2+ (Nadler, et al., 1981b; Friedman, et al., 1985) and presumably belong to a "less mature" subgroup by phenotypic criteria. However, the precise clinical significance of the expression or absence of B2 in histologically defined B cell tumours is presently unknown.

The weak expression of Leu1 (OKT1) in case 9 suggests that at least a proportion of the lymphoma cells in this case were of a less mature B cell category than cases 4 and 6 i.e., similar to B-CLL (Anderson, et al., 1984).

The lack of overlap between B1/B2 and T cell markers (Table 8) i.e., lack of "double marker" expression does suggest B1 (and to a lesser extent B2) will be useful in differentiating B cell from T cell derived tumours.

9) Follicular (Nodular) Lymphoma

The results in case 9 are of particular interest as there are few reports to date of McAb phenotyping of cutaneous nodular (follicular) lymphoma.

Early observations suggested that lymphomas with a follicular pattern are related to and possibly derived from normal lymphoid follicles (Brill, et al., 1925; Symmers, 1927).

The term "nodular" later replaced the term "follicular" in describing these tumours as Rappaport (Rappaport, et al., 1956) argued that the follicular pattern did not prove a relationship to normal follicles but might merely reflect a cohesive tendency of the cells. However, light microscopic, (Lukes and Collins, 1974) electron microscopic (Levine and Dorfman, 1975), and immunologic studies (Jaffe, et al., 1974) have provided further evidence for the relationship

between nodular lymphomas and normal lymphoid follicles (LFs) and the term "follicular" has regained acceptance. Despite the presence of pseudonodular patterns in T cell lymphoma (Ioachim and Finkbeiner, 1980) and negative staining patterns for SIg in some cases of follicular lymphoma (Garcia, et al., 1986) it is, therefore, generally accepted that nodular lymphomas are B cell derived tumours. Testing of case 9 shows many similarities between the lymphoma follicles and non-malignant lymphoid tissue (Chapter 4) strengthening this concept. This is illustrated in Table 11.

The main differentiating features in follicular lymphoma are: 1) The presence of Be1+, Be2+, GC cells, monoclonal K/L ratio, weak Leu1 GC expression and lack of My13+ GC cells in LFs. 2) Tendency to Leu2A+ (suppressor) preponderance, large numbers of HNK1+ lymphocytes, and occasional "spillage" of B1+ and R423+ cells in IFAs.

Follicular dendritic reticulum cells (DRC) are identified in case 9 with McAb R423. These are absent in the other lymphomas (including 2 diffuse B cell) examined.

In 1927 Maximow described a non-lymphoid population of embryonic non-phagocytic reticulum cells (Maximow, 1927) ("embryonale nicht speichernde Retikulumzellem") which are present in cortical follicles. Later, electron microscopic studies (Swartzendruber, 1965) and immunologic studies (Nossal, et al., 1968) indicated these cells are restricted to cortical follicles.

R423+ cells may also be found in the LFs of benign cutaneous B cell infiltrates (Chapter 7). The B cell monoclonal antibody B2 may also be reacting with DRC in case 9 as in normal lymphoid tissue (Chapter 4). (The B2+ cells in case 4 are presumably B cells as a negative reaction is obtained with R423.)

Some diffuse B cell lymphomas (centrocytic by Kiel classification) may also contain R423+ DRCs (Stein, et al.,

1982; Naiem, et al., 1983).

Electron microscopic studies in the early 1970's by Kojima (Kojima, et al., 1973) and Levine (Levine and Dorfman, 1975) demonstrated the presence of DRCs in nodular lymphoma. Levine et al. (Levine and Dorfman, 1975) suggested that they merely represented residual markers of pre-existing germinal centres. Kojima et al. (Kojima, et al., 1973) on the other hand regarded DRCs as lymphoid cell precursors and nodular lymphomas as dendritic cell neoplasms.

Radioautographic studies certainly do not support the concept of DRCs as lymphoid precursors (Everett and Caffrey, 1967). However, the demonstration of R423+ DRCs in extra-nodal lymphoma in case 9 suggests these cells may be intimately involved in the formation of the lymphoid follicles and possibly in the migration of the neoplastic process. DRCs certainly appear in neonatal rat lymph node cortex prior to the development of recognizable primary follicles and germinal centres (Nossal and Ada, 1971). The lack of demonstrable antibody and complement on the DRC of neoplastic follicles (Stein, et al., 1982), however, suggests that such a "B cell trap" in neoplastic and possibly normal follicles is unlike antigen trapping and probably antibody and C3 independent (Stein, et al., 1982).

Testing in case 9 with the anti-CALLA reagent J5 is negative when used in a concentration similar to that used originally in determining its distribution in normal tissues (Ritz, et al., 1980; Metzgar, et al., 1981) and CTCL (Chapter 5) with the indirect immunoperoxidase technique. The presence of CALLA has been shown to correlate with a follicular architecture (Garcia, et al., 1986). The negative reaction in case 9 indicates that CALLA in high concentration is not a sine qua non of follicular architecture in cutaneous lymphomas.

The significance of weak Leu1 expression in some of the

lymphoma cells in case 9 is unknown. A similar phenomenon has been noted in nodal follicular lymphomas by some groups (Knowles, et al., 1982; Burns, et al., 1983) but not by others (Harris, et al., 1984). Among B cell neoplasia the Leu1 (T1) antigen is formed most commonly on B-CLL (Royston, et al., 1980). Its expression on B cell lymphomas may represent aberrant antigen expression by neoplastic B cells or derivation from an uncommon Leu1 (T1) positive normal B cell (Caligaris-Cappio, et al., 1982).

Ree et al. (Ree and Leone, 1978) demonstrated a positive correlation between the prominence of perifollicular lymphocyte rims which consist predominantly of non-neoplastic B-lymphocytes (Harris and Data, 1982), and survival in nodular lymphoma. Colby et al. (Colby, et al., 1980) on the other hand could not confirm this but found such a correlation between survival and degree of nodularity (Colby, et al., 1980). The presence of Leu1+, OKT3+ and HNK1+ lymphocytes in the interfollicular areas of case 9 suggests that similar studies of these associated putative immune cells would be of interest.

10) Common Leukocyte Antigen 2D1

The monoclonal antibody 2D1 may have application in differentiating lymphomas from epithelial malignancies and sarcomas (Pizzolo, et al., 1980). All 13 lymphomas are positive suggesting this reagent might be useful in "null" cases where marker expression is insufficient for even broad inferences to be made as to the neoplastic cell sub-type. However, further information is required concerning the sensitivity of this marker as 2 of 20 lymphoid malignancies in Pizzolo's original study were 2D1 negative (Pizzolo, et al., 1980).

11) Class I and II HLA Expression

Five of five cases tested (Numbers 2,3,4,9,10) are class 1 HLA+. This contrasts with one study of three diffuse histiocytic lymphomas which were HLA- (Woda, et al.,

1981). Four of four cases tested are Class II HLADR+ (Ia+). This contrasts with MF which generally shows an HLADR- phenotype (Wood, et al., 1982).

12) Myeloid Antibodies

The results obtained with the myeloid antibodies indicate the frequent presence of macrophage subpopulations bearing the My3 and My13 determinants as in non-malignant lymphoid tissue (Chapter 4). The My11+ phenotype in 4 of 5 cases (Numbers 2,3,4,9) fits with the broad spectrum of reactivity of this antibody which reacts with E+ and E- PBL.

The negative reaction in 5 of 5 cases with MY10 and My12 parallels the results with these reagents in non-malignant lymphoid tissue and is consistent with their restricted specificity for immature myeloid cells.

13) Macrophages (Esterase, LeuM1, LeuM3)

Esterase positive macrophages are identified in 5 of 5 T cell, 2 of 2 B cell, and 1 of 1 "U" cell lymphomas. Macrophages are also readily identified in T, B and U cell lymphomas with the use of the LeuM1/LeuM3 McAbs, although these show varying degrees of positivity. The degree of overlap between LeuM1, LeuM3, and esterase positive cells is unknown.

The differing distributions of LeuM1 and LeuM3 positivity in non-malignant lymphoid tissue (Chapter 4) and follicular B cell lymphoma (Table 11) and disparate reaction in case 6 (LeuM1-, LeuM3+) (Table 8) suggests the presence of discrete LeuM1+, LeuM3+ subpopulations.

On the basis of light and electron-microscopic studies in 7 diffuse T cell lymphomas Palutke et al. described numerous admixed macrophages as a characteristic feature of T cell lymphomas of large cell type (Palutke, et al., 1980). The results here indicate that macrophages are frequently found in lymphomas of T, B and U types and their presence per se is not an accurate predictor of neoplastic cell lineage.

TABLE 11-COMPARISON OF IMMUNO-ARCHITECTURAL PATTERN IN FOLLICULAR LYMPHOMA (FL) AND NON-MALIGNANT LYMPHOID TISSUE (NMLT)

A. GERMINAL CENTRES	FL	NMLT
1. B1+ phenotype	+	+
2. B2+ cells rounded pattern	+	+
3. B2+ dendritic pattern extending into MZ	+	+
4. Ia+ (HLADR+) phenotype	+	+
5. OKT9+ cells (transferrin receptor)	+	+
6. OKT10+ cells (occasional)	+	+
7. CALLA+ (J5+) phenotype	-	-
8. OKT6+ cells	-	-
9. Esterase+ macrophages	+	+
10. M1+ cells	-	-
11. M3+ cells	+	+
12. R423+ follicular dendritic cells	+	+
13. T cells (OKT3+ T11+)	+	+
14. Leu3A+ helper T cells (H>S)	+	+
15. Leu2A+ suppressor T cells	+	+
16. Be1+ cells	+	-
17. Be2+ cells	+	-
18. Polyclonal K/L ratio	-	+
19. Polyclonal mantle zone (variable)	+	+
20. HNK1+ lymphocytes	+	+
21. Weak expression of Leu1 on B cells (variable)	+	-
22. My3+ dendritic cells	+	+
23. HLA+ phenotype	+	+
24. 2D1+ (human leukocyte antigen) phenotype	+	+
25. My10+ cells	-	-
26. My11+ phenotype	+	+
27. My12+ cells	-	-
28. My13+ cells	-	+
B. INTERFOLLICULAR AREAS		
1. Preponderance of T cells (OKT3+ T11+ Leu1+)	+	+
2. Preponderance of Helper T cells (Leu3A)	-	+
3. "Spillage" of B1+ cells from LFs	+	-
4. B2+ cells	-	-
5. Ia+ cells	+	+
6. OKT9+ cells	+	+
7. CALLA+ J5+ cells	-	-
8. OKT6+ cells (in tonsil)	-	-
(in occasional Lymph nodes)		+
9. Esterase+ macrophages	+	+
10. M1+ cells	+	+
11. M3+ cells	-	-
12. R423+ cells (rare + cells in FL)	-	-
13. HNK1+ lymphocytes	+	-
14. OKT6+ cells in adjacent epithelium	+	+
(FL + [resident epidermal cells])		
(NMLT + [tonsillar crypt epithelium)		
15. Exocytic T cells in adjacent epithelium	-	+

14) Be1 and Be2 (Table 8)

Be1 and Be2 reactivity (5/11 and 6/11 cases respectively), are not confined to any particular histologic or phenotypic category of tumour. The results indicate in addition that only a proportion of lymphomas react with these reagents.

15) HNK1

HNK1+ lymphocytes are identified in 6/12 tumours (range <1%-10% of cells). They are not confined to any lineage, being present in 3 T, 2 B, and 1 U cell, and are present in all histologic categories. The results, therefore, confirm that HNK1+ lymphocytes may be found in situ in human lymphomatous lesions.

The lymphomas are non-epidermotropic and exocytic HNK1+ lymphocytes are absent from positively staining specimens. HNK1+ lymphocytes are distributed apparently at random throughout the superficial and mid-reticular dermal infiltrates. No clustering of HNK1+ lymphocytes is observed. In the nodular CBCL HNK1+ lymphocytes are distributed mainly between the pseudofollicles and within areas showing a diffuse pattern of proliferation.

The largest number of HNK1+ lymphocytes are observed in the case of nodular CBCL (10% of infiltrating cells). This may reflect the normal propensity of these cells for lymphoid follicles (Chapter 4). However, the contrast in distribution observed between nodular CBCL and tonsillar lymphoid follicles (Table 11) suggests that this explanation is overly simplistic. The distribution of the HNK1+ lymphocytes in the follicular lymphoma (Plate 51) is, therefore, also compatible with a host-tumour interaction.

16) Unclassifiable (U Cell) Types

Although inferences can be made concerning lineage in "U cell" lymphomas (cases 1 and 8) cases of NHL have been reported which are totally devoid of any lineage related markers, reacting only with Ia (Wood, et al., 1983b), OKT10,

(Aisenberg and Wilkes, 1980; Aisenberg, et al., 1983) or OKT9 and OKT10 (Aisenberg, et al., 1983). Batteries of McAbs may, therefore, not be a panacea in neoplastic cell categorization and may require complementary functional studies and simultaneous testing for clonal gene rearrangements.

CHAPTER SEVEN

MISCELLANEOUS CUTANEOUS LYMPHOHISTIOCYTIC INFILTRATES

Including:

A. Introduction
B. Materials and Methods
C. Results
    1. Lymphocytoma Cutis (Pseudolymphoma)
    2. Lymphomatoid Papulosis (Types A and B) (Pseudolymphoma)
    3. Lymphocytic Infiltrate of Jessner (Pseudolymphoma)
    4. Sarcoidosis (Benign Histiocytic Infiltrate)
    5. Histiocytosis X. (Malignant Proliferation of True Histiocytes)
    6. Chronic Lymphocytic leukaemia (Leukaemia Cutis)
    7. Myelomonocytic Leukaemia (Leukaemia Cutis)
D. Discussion
    1. Lymphocytoma Cutis
    2. Lymphomatoid Papulosis
    3. Lymphocytic Infiltrate of Jessner
    4. Sarcoidosis
    5. Histiocytosis X
    6. Chronic Lymphocytic Leukaemia
    7. Myelomonocytic Leukaemia

CHAPTER SEVEN

MISCELLANEOUS CUTANEOUS LYMPHOHISTIOCYTIC INFILTRATES

A. INTRODUCTION

No study of immunological markers in lymphoma would be complete without a comparable study of a) cutaneous pseudolymphomas, eg., lymphocytoma cutis (From, 1979), lymphomatoid papulosis (Macaulay, 1968), lymphocytic infiltrate of Jessner (From, 1979), b) benign infiltrates with a marked histiocytic component (eg. sarcoidosis), c) histiocytic malignancy (eg. histiocytosis X), and d) leukaemia cutis.

A variety of the above disorders are, therefore, included.

B. MATERIALS AND METHODS

The following disorders are studied:

Lymphocytoma cutis (3 cases), lymphomatoid papulosis type B (Willemze, et al., 1982b) (1 case), lymphomatoid papulosis type A (Willemze, et al., 1982b) (2 cases), lymphocytic infiltrate of Jessner (LIJ, 1 case), sarcoidosis (1 case), histiocytosis X (3 cases), chronic lymphocytic leukaemia (CLL, 1 case) and myelomonocytic leukaemia (Naegeli Type) (MML, 1 case). The lymph node of 1 case of lymphomatoid papulosis type A which eventuated in nodal diffuse histiocytic (large cell immunoblastic polymorphous) lymphoma is also studied and compared with the cutaneous phenotypic pattern.

The reagents used include Leu1, Leu2A, Leu3A, OKT6, OKT9, OKT10, Leu14, B1, B2, HLADR, R423, LeuM1, LeuM3, HNK1, Anti-Kappa, Anti-Lambda, Anti-Leuk 2D1, Multiclone T, Leu4/5, and non-specific esterase.

In Histiocytosis X, J5, MY10 and My11 are also tested in the first 2 cases. Because of limited amounts of tissue, Case 3 is only tested with OKT6, LeuM1, LeuM3 and HNK1.

C. RESULTS

1. Lymphocytoma Cutis (From, 1979) (cutaneous lymphoid

hyperplasia, Spiegler-Fendt sarcoid, Type 4 cutaneous lymphocytic infiltrate).

The 3 cases examined show clusters of B1/B2/Leu14 positive B cells (Plate 53) surrounded by Leu1+ T cells showing a mixture of Leu3A+, Leu2A+ cells (H/S ratio 1:1 to 3:1). The B cell clusters are HLADR positive and contain interspersed R423 positive dendritic cells (Plates 54, 55). LeuM3+ macrophages are present within the lymphoid follicles in 3 cases and LeuM1+ cells are found scattered throughout the infiltrates in 2/3 cases. OKT9 positive cells are prevalent in LF of 2/2 cases tested. Esterase positive macrophages are present within LF (Plate 56) and in an extrafollicular location. Leu1+, Leu2A+, Leu3A+ cells are present within LFs and in a perifollicular location, although, a crescentic Leu3A pattern as seen in tonsillar secondary LFs is not seen. Epidermal dendritic OKT6 positive cells are present in all 3 cases, apparently in a normal distribution. Exocytic Leu1+ cells are absent. The epidermal OKT6/Leu1 pattern, therefore, fits with the non-epidermotropic pattern found in this group of disorders and contrasts with that found in acute allergic contact dermatitis (Chapter 4). Dermal dendritic OKT6 positive cells are found in only 1 of 3 cases and these are in a perifollicular location, similar to lymph node (Chapter 4). The Kappa/lambda ratio, measured in 2 cases, is 1:1 and 4:1.

HNK1+ lymphocytes are present in 1/2 cases. These are in a follicular location as in non-malignant lymphoid tissue (tonsil).

2. Lymphomatoid Papulosis (Type 6 cutaneous lymphocytic infiltrate. (From, 1979)

Because of the diverse patterns observed, these cases will be described separately.

Case 1. Lymphomatoid papulosis type B. (Willemze, et al., 1982b)

The majority of large atypical cells (LAC) are Leu1+,

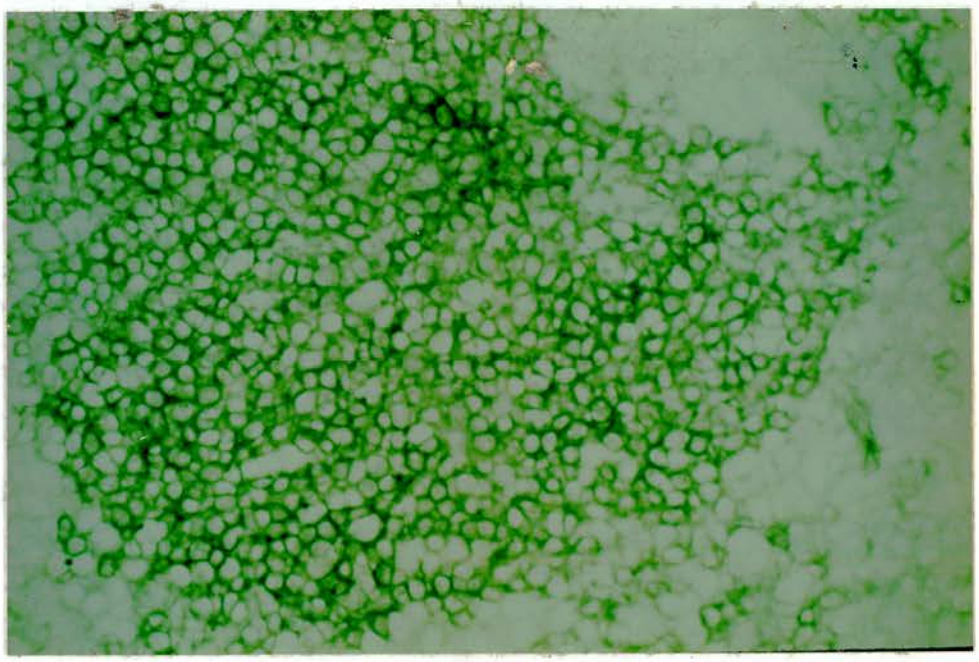


PLATE 53. **LYMPHOCYTOMA CUTIS** (Spiegler Fendt Sarcoid, Cutaneous lymphoid Hyperplasia). Lymphoid follicle contains large number of Leu14+ B cells. Indirect immunoperoxidase. Non counterstained. Green filter x 100.

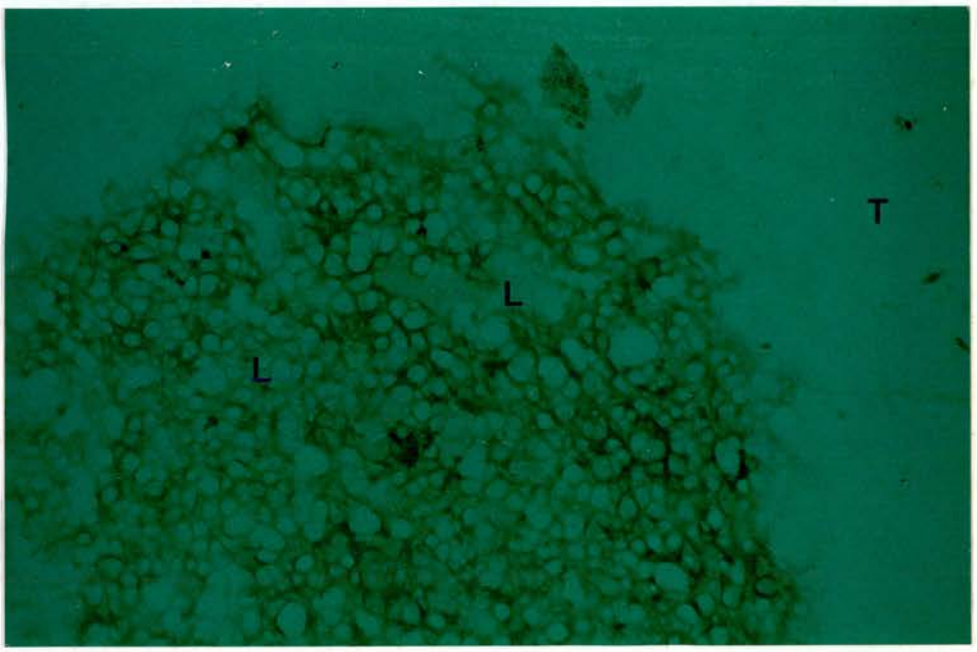


PLATE 54. **LYMPHOCYTOMA CUTIS.** Lymphoid follicle (L) contains large number of R423+ cells forming a dendritic meshwork. Surrounding dermal T cell infiltrate (T) stains negatively. Indirect immunoperoxidase. Non counterstained. Green filter x 100.

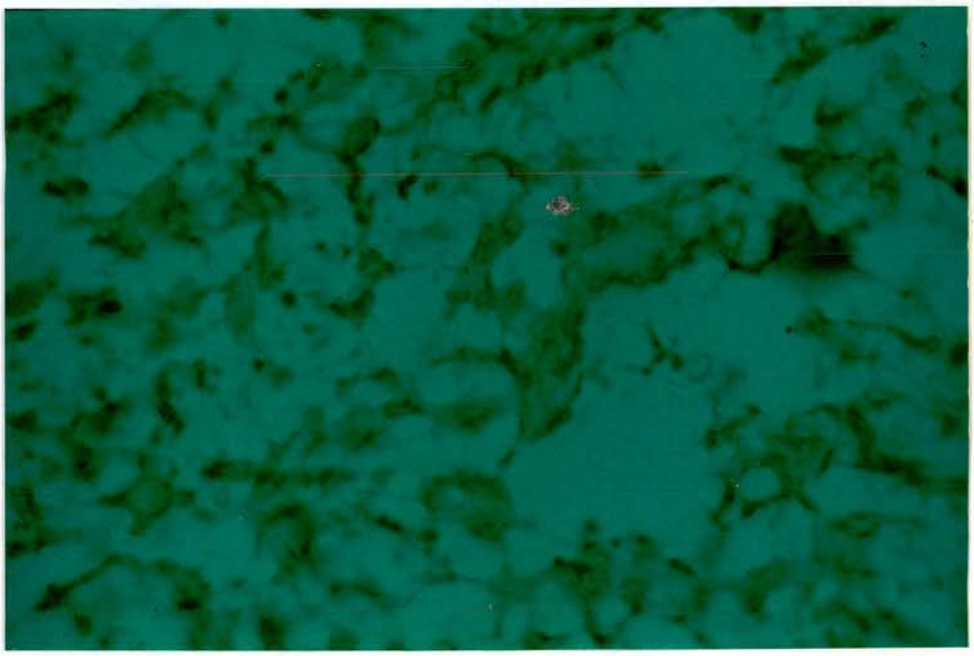


PLATE 55. **LYMPHOCYTOMA CUTIS.** High power view of R423+ dendritic cells interspersed throughout follicular B cells. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

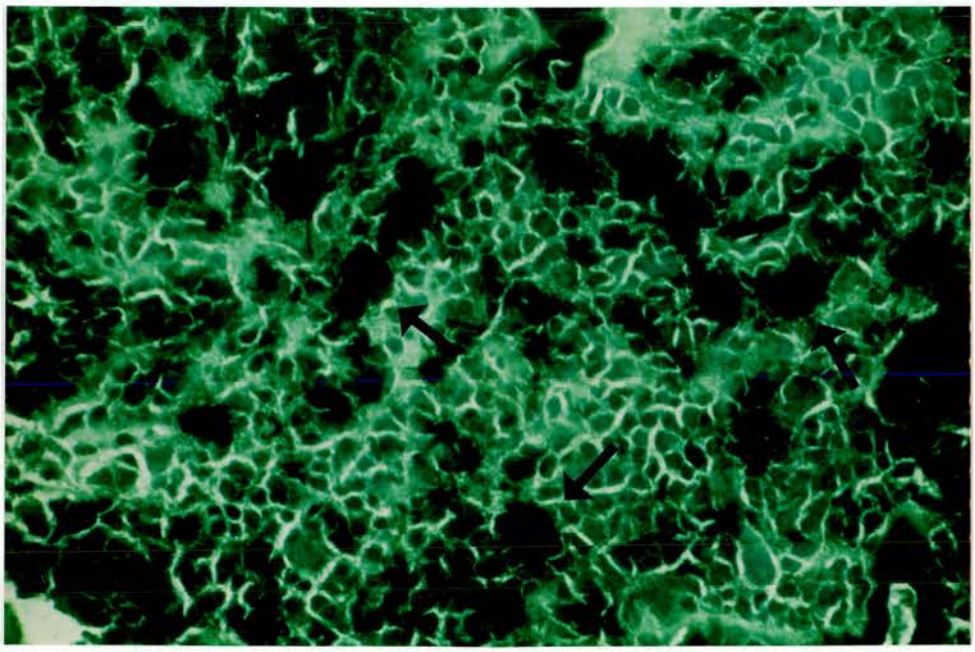


PLATE 56. **LYMPHOCYTOMA CUTIS.** Esterase positive macrophages (arrows) among follicular lymphoid cells, similar to non malignant lymphoid tissue Alpha Naphthyl Acetate Esterase. Methyl green counterstain. Green filter x 100.

Leu3A+. Throughout the lymphoid population helper preponderance is present (H/S. 3:1). Dermal OKT6 dendritic cells are interspersed throughout. (Approximately 10% of infiltrating cells). Large cells with diffuse cytoplasmic esterase activity form approximately 10% of the population. LeuM1 and/or LeuM3 positive macrophages are found scattered throughout (LeuM1/LeuM3, 3:1). HNK1+ lymphocytes form less than 1% of the infiltrating cells. A negative reaction is obtained with OKT9, B1, B2, R423.

Case 2. Lymphomatoid Papulosis type A. (Willemze, et al., 1982b)

The majority of LAC are Leu1- and show variable strengths of diffuse cytoplasmic esterase (Plate 57) and Leu3A positivity. These cells are strongly HLADR positive. This immunochemical pattern is also present in the Reed-Sternberg (RS) like cells (Plate 57) so typically seen in this subgroup. The lymphoid component is Leu1+ and exhibits variable HS ratios. (H/S 1:1 in an 8 day old papule and H/S 1:3 in an 8 week old papule). HNK1+ lymphocytes form 10% of the lymphoid population in the 8 week old lesion. A negative reaction is obtained with OKT9, OKT10, B1/B2, and R423.

Case 3. Lymphomatoid papulosis type A.

A mixture of patterns is seen in the large cell component with Leu1+, Leu3A+ cells preponderant in early biopsies (H/S, 3:1), with an admixture of Leu1- cells with diffuse-cytoplasmic esterase and variable cytoplasmic Leu3A positivity. OKT6 positive cells form approximately 10% of the infiltrate. HNK1+ cells form <1% of the total infiltrating cells. Large clusters of cells reacting only with OKT9 are also present, reminiscent of the pattern seen in CTCL (Chapter 5).

In later biopsies approximately 80% of the cellular population reacts with OKT9 and HLADR (Plate 58) alone, with large clusters of OKT6 positive cells (Plate 59).

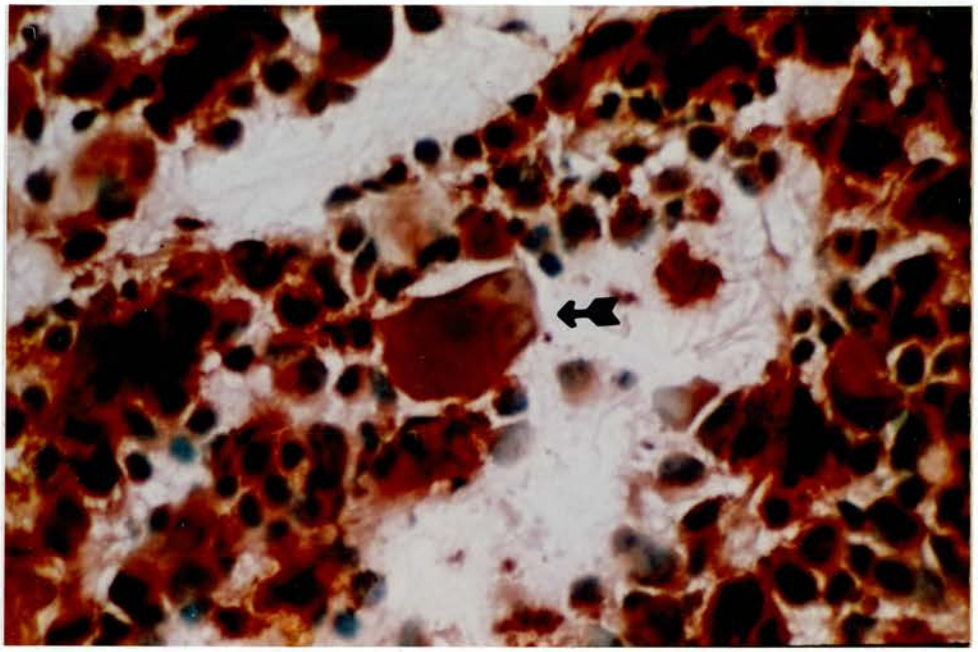


PLATE 57. **LYMPHOMATOID PAPULOSIS TYPE A.** Binucleate Reed Sternberg-like cell centre of field (arrow) exhibits diffuse cytoplasmic esterase activity. Alpha Naphthyl Acetate Esterase. Methyl green counterstain x 160.

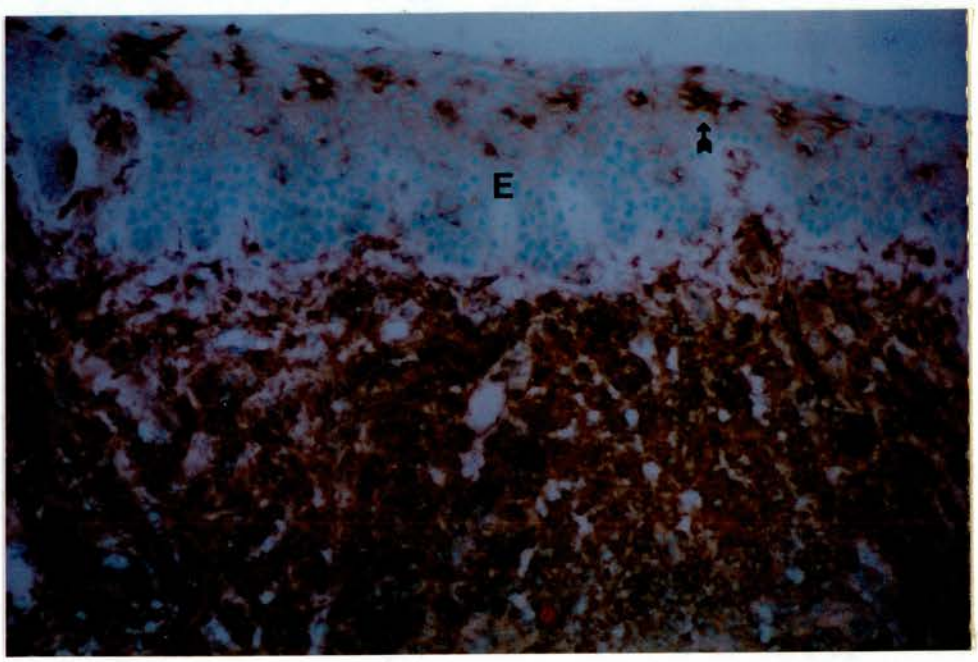


PLATE 58. **LYMPHOMATOID PAPULOSIS TYPE A.** HLADR+ dermal infiltrate. Dendritic cells (arrow) within epidermis (E) are also HLADR+. Indirect immnoperoxidase. Haematoxylin counterstain x 100.

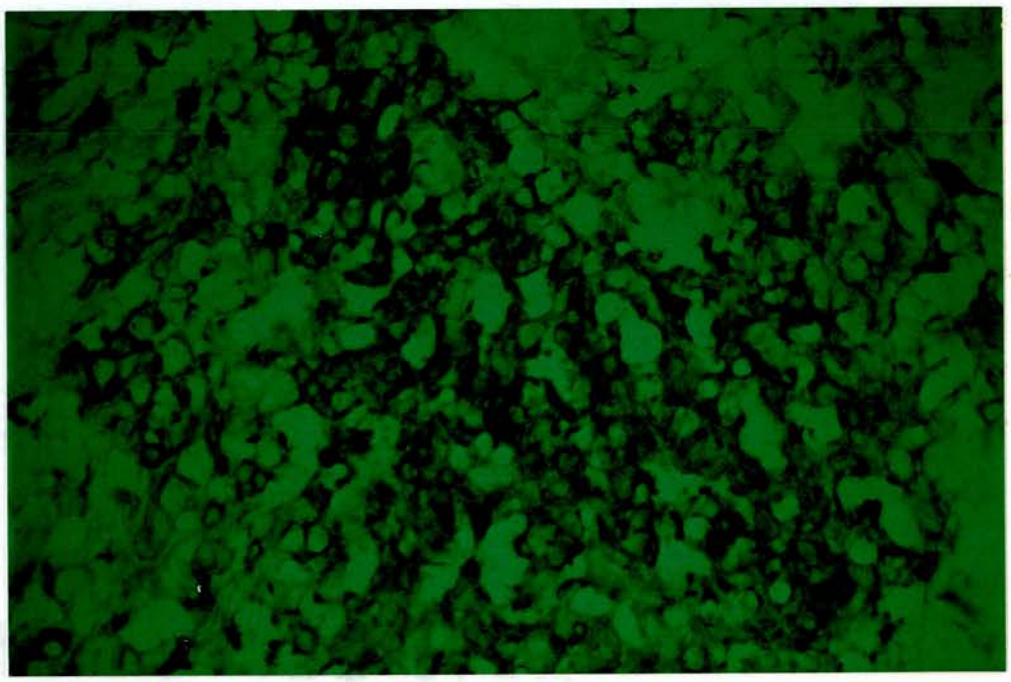


PLATE 59. **LYMPHOMATOID PAPULOSIS TYPE A.** Cluster of OKT6+ dermal cells. This pattern is apparently uncommon in type A lesions. Indirect immunoperoxidase. Non counterstained. Green filter x 100.

A negative reaction is obtained throughout with OKT10, B1/B2, and R423.

LeuM1 and/or LeuM3 positive macrophages are occasionally found (<10% of infiltrate, LeuM1/LeuM3, 1:2).

After four years of recurring self healing cutaneous papules, plaques, and nodules this patient developed a nodal lymphoma. The pathology will be discussed later. Phenotyping of the nodal infiltrate shows the neoplastic population to be Leu1-, Leu3A-, OKT6-, OKT9+, B1-, B2-, 2D1+, HLADR+, R423-, LeuM3-, HNK1-, NSE-, multiclone T+ (Leu4/5+). Clusters of large LeuM1+ cells are also present (<10% of infiltrating cells.)

3. LYMPHOCYTIC INFILTRATE OF JESSNER (Type 3 cutaneous lymphocytic infiltrate) (From, 1979).

Testing shows a dermal Leu1+ population (H/S 4:1). Occasional OKT10+ cells are observed (10% of the population). Although epidermal OKT6+ cells are present, there is a complete absence of dermal OKT6+ cells. HLADR positivity is present throughout the lymphoid population. A negative reaction is obtained with B1/B2, R423 and HNK1.

4. SARCOIDOSIS

The epithelioid histiocytes of sarcoidosis are esterase+, LeuM3+, LeuM1-, OKT6-, OKT9+ (Plates 60, 61). Associated lymphoid cells are Leu1+, Leu3A+ (H/S > 5:1) with Leu3A+ lymphocytes scattered throughout the granulomas and Leu2A+ cells tending to be found at the periphery. HNK1+ lymphocytes are absent.

5. HISTIOCYTOSIS X

The results in Histiocytosis X are summarized in Table 12. The HX tumour cells are OKT6+, HLADR+ with variable Leu3A positivity (Plates 62, 63, 64, 65).

6. CHRONIC LYMPHOCYTIC LEUKAEMIA

The leukaemic infiltrate is not tested with a full range of T+B markers. However, the infiltrating cells are

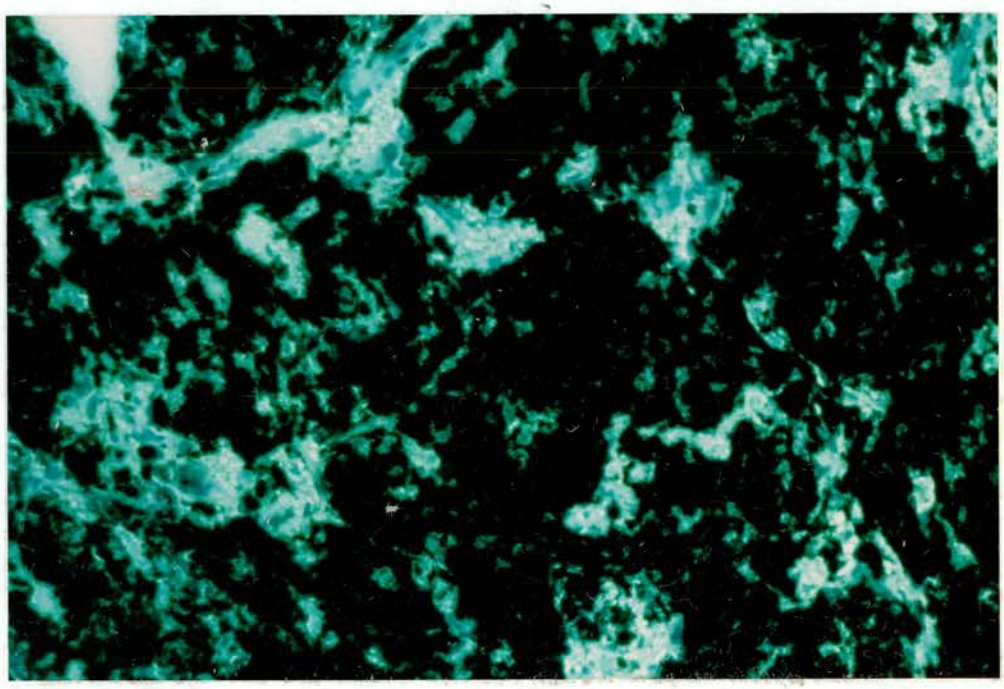


PLATE 60. **SARCOIDOSIS.** Epitheloid histiocytes are esterase positive. Alpha Naphthyl Acetate Esterase. Methyl green counterstain. Green filter x 40.

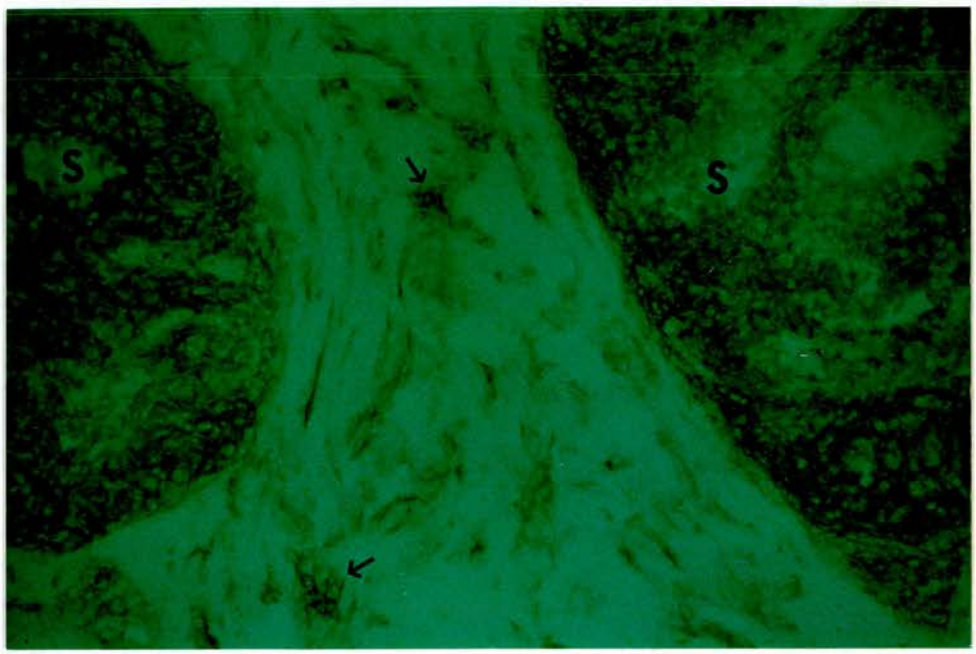


PLATE 61. **SARCOIDOSIS.** Epithelioid histiocytes of sarcoidal granulomas (S) are LeuM3+. Scattered LeuM3+ histiocytes (arrow) are also present between the granulomas. Indirect immunoperoxidase. Non counterstained. Green filter x 40.

TABLE 12-IMMUNOPHENOTYPE OF HISTIOCYTOSIS X

	Preponderant phenotype of histiocytic cells (>50% staining positively)	Additional positively staining histiocytes (≤50% staining positively)	Reactions observed in small mononuclear component (mainly lymphocytes)	Negative reactions
Case 1 (scalp, truncal)	OKT6+(>80%), HLADR (>70%)	Leu3A (50%), OKT9 (<50%)	Leu 1, OKT3, (>70%)	Leu2A, OKT10, B2, J5, HNK1, LeuM1, LeuM3, R423, My10, My11
Case 2 (scalp)	OKT6+ (80%) HLADR+ (65%), Leu3A+ (65%)	OKT9 (10%), My10 (<10%)	Leu 1 (>70%), OKT3 (>70%), OKT10 (<10%), HNK1 (1%) LeuM1(<10%), LeuM3 (<10%), My11 (>70%)	B1, B2, J5, R423
Case 3 (eyelid)	OKT6+	- - -	LeuM1, LeuM3 (35%), HNK1 (<1%)	- - -

Note: In Case 1, the infiltrating nodal sinusoidal histiocytes, which on light microscopy had morphology characteristic of HX cells, had an OKT6+ HLADR+ Leu3A+ phenotype. A few scattered residual lymphoid cells reacting with lymphocyte monoclonals (Leu1, Leu2A, Leu3A etc.) were also noted in the lymph node. HNK1+ lymphocytes were not observed. Scalp and truncal biopsies in Case 1 showed similar phenotypes. Both scalp biopsies in Case 2 showed similar phenotypes. Case 3 was only tested with OKT6. LeuM1, LeuM3 because of limited amounts of tissue available. Percentages expressed are approximations. Accurate percentages were not possible because of the varying strength of staining observed and the apposition of cells in dense infiltrates. Estimation of the proportion of cells expressing the Leu 3A determinant was particularly difficult because the staining intensity with this antibody was more variable and less intense than with HLADR and OKT6. Figures are expressed as a percentage of histiocytes or small mononuclear cells, not as a percentage of the total cell population.

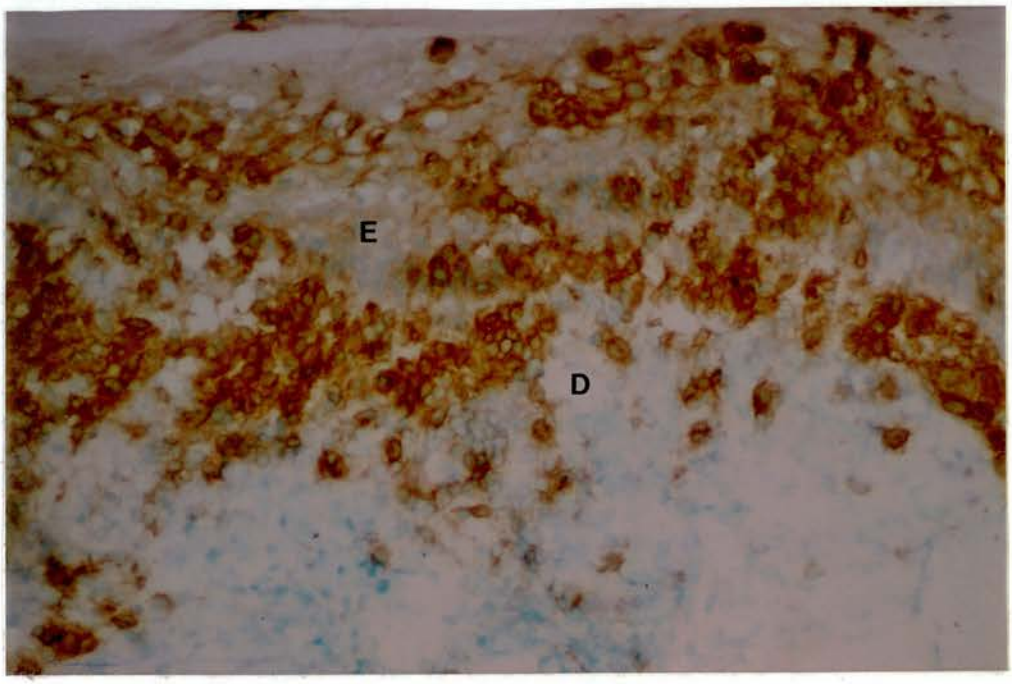


PLATE 62. **HISTIOCYTOSIS X (SKIN).** OKT6+ histiocytes in upper dermis, at dermo-epidermal junction and infiltrating epidermis. Epidermis (E). Dermis (D). Indirect immunoperoxidase. Haematoxylin counterstain x 64.

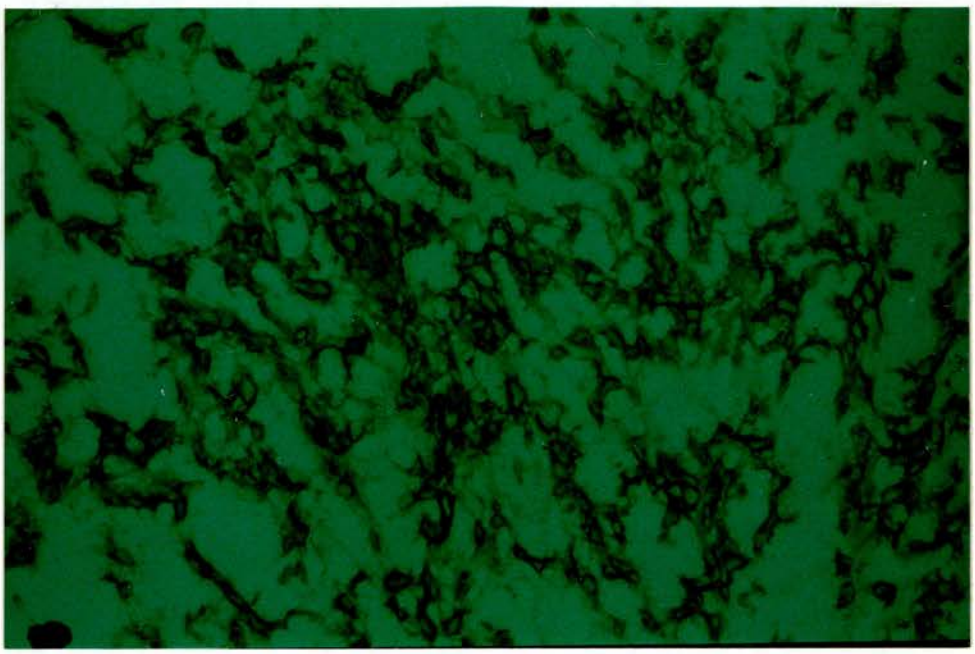


PLATE 63. **HISTIOCYTOSIS X (SKIN).** Morphology of OKT6+ cells varies. In this tumour the histiocytosis cells are dendritic. Indirect immunoperoxidase. Non counterstained x 64.

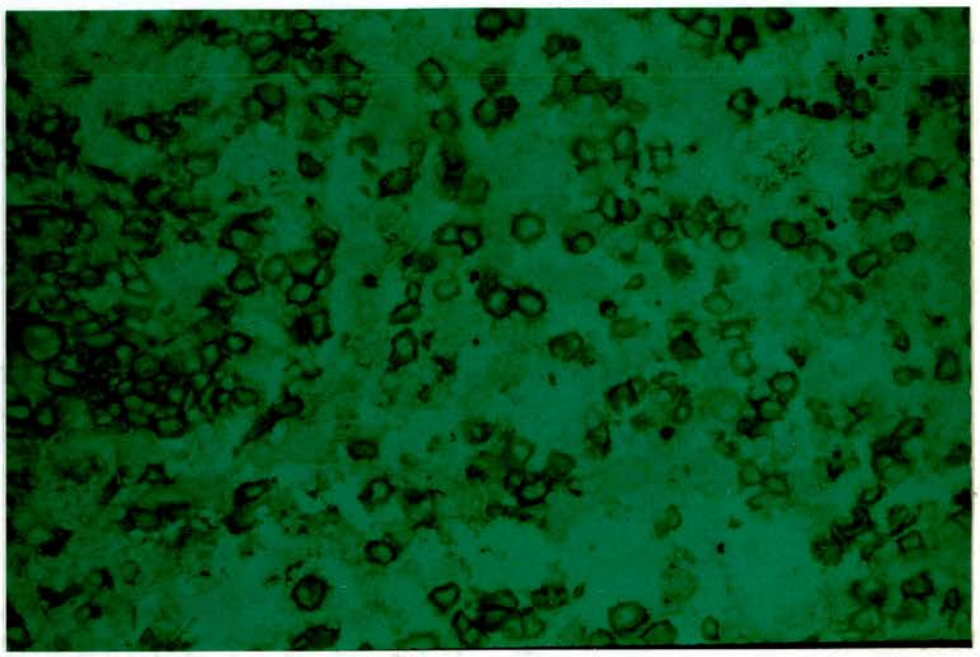


PLATE 64. **HISTIOCYTOSIS X. (PERIORBITAL TUMOUR).** Although many OKT6+ tumour cells are dendritic, the morphology is clearly more rounded than in tumour in Plate 63. Indirect immunoperoxidase. Non counterstained. Green filter x 64.

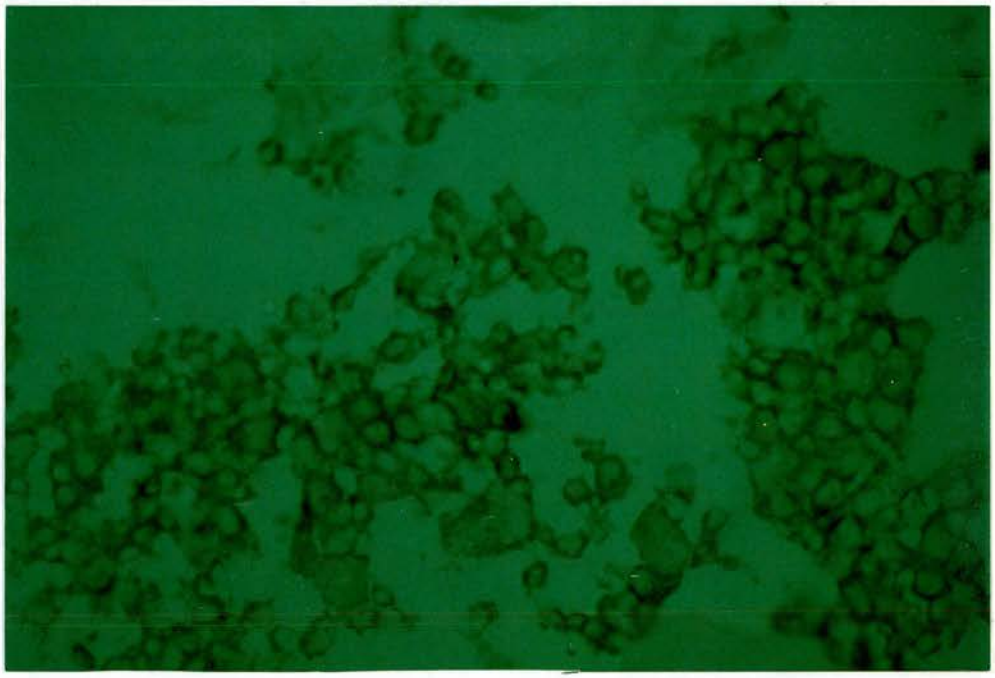


PLATE 65. **HISTIOCYTOSIS X (LYMPH NODE).** Tumour cells are Leu3A+. This antigen (normally T helper) is more readily identified on histiocytosis X cells than normal Langerhans' cells. Indirect immunoperoxidase. Non-counterstained. Green filter x 100.

Leu1+ (Plate 66) (H/S 2:1). An unusual feature in CLL is an absence of OKT6 positive epidermal dendritic cells above the infiltrated area of skin.

7. MYELOMONOCYTIC LEUKAEMIA (Plates 67, 68)

The leukaemic infiltrate is LeuM1+, OKT9+. A proportion of the infiltrating cells are LeuM3+ (LeuM1/LeuM3, 4:1). A negative reaction occurs with Leu1, Leu2A Leu3A, OKT6, B1/B2, NSE, R423, and HNK1.

The results are summarized in Table 13.

D. DISCUSSION

1. LYMPHOCYTOMA CUTIS (TABLE 13)

The B cell nature of lymphocytoma cutis has previously been shown in immunological (Braun-Falco and Burg, 1975) and ultrastructural (Schmoeckel, et al., 1977) studies.

The clustering of B1+/B2+ cells with interspersed R423 (follicular dendritic) cells and LeuM3+ NSE+ macrophages intermixed with and surrounded by Leu1+, Leu2A+/Leu3A+ T lymphocytes (Figure 8) is remarkably similar to the situation found in lymphoid follicles of nodal (Figure 2) and tonsillar tissue (Figure 1). The presence of perifollicular OKT6+ dendritic cells in 1 case is also similar to that occasionally found in nodal tissue (Chapter 4). Although OKT6+ dendritic cells are rare in the perifollicular regions of tonsil (Chapter 4) analogies can also be drawn between that organ and skin affected by lymphocytoma cutis when one considers the presence of LFs adjacent to OKT6+ dendritic cells in crypt epithelium of tonsil (Figure 1)(Chapter 4) and the presence of resident OKT6+ LC in overlying epidermis in lymphocytoma cutis (Figure 8).

The role of skin as a lymphoid organ has been previously postulated (Fichtelius, et al., 1970) and the term Skin Associated Lymphoid Tissue (SALT) (Streilein, 1978) has been coined. Various antigens have been incriminated in lymphocytoma cutis, including viruses

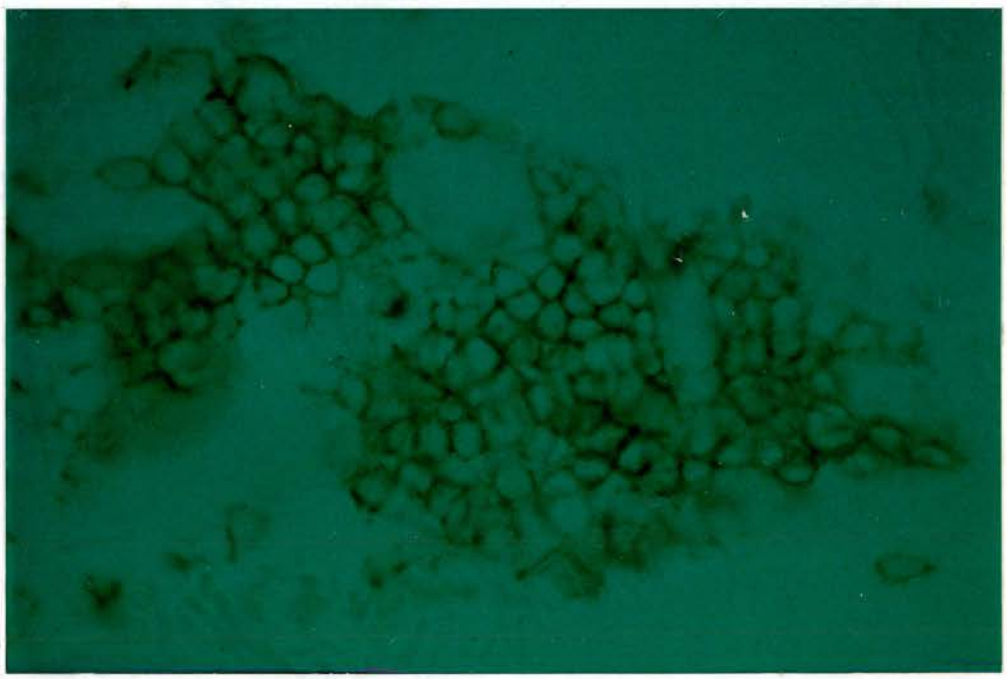


PLATE 66. **CHRONIC LYMPHOCYTIC LEUKAEMIA.** Leu1+ perivascular dermal infiltrate. Indirect immunoperoxidase. Non counterstained. Green filter x 160.

PLATE 67. **MYELOMONOCYTIC LEUKAEMIA.** Cutaneous papules and plaques.

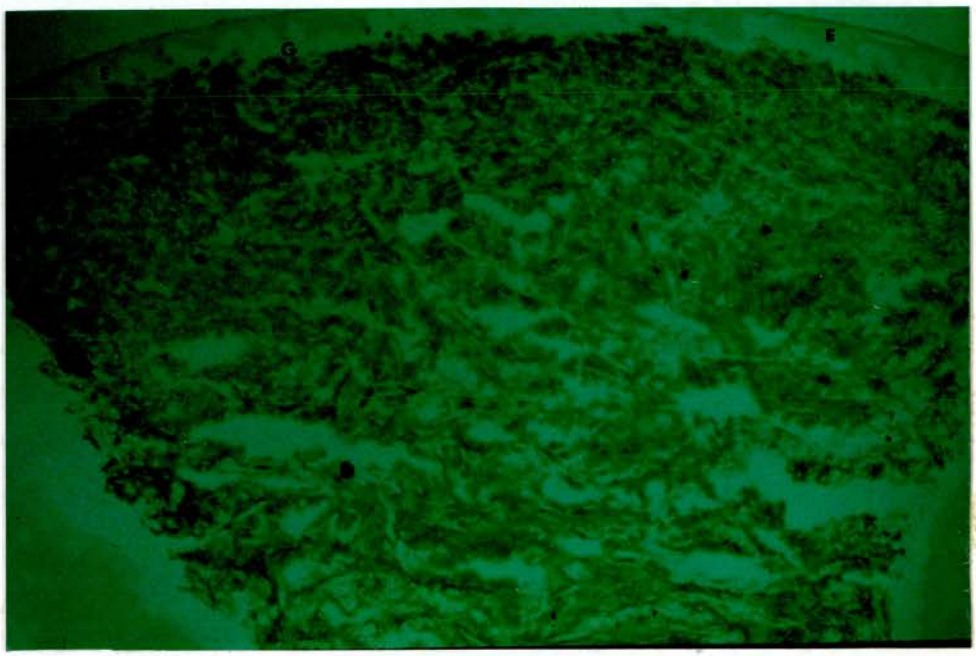


PLATE 68. **MYELOMONOCYTIC LEUKAEMIA (SKIN).** Leukaemic infiltrate, which occupies full thickness of reticular dermis apart from Grenz zone (G), is LeuM1+. Epidermis (E). Indirect immunoperoxidase. Non counterstained. Green filter x 10.

TABLE 13-MONOCLONAL ANTIBODY PATTERNS IN MISCELLANEOUS DERMAL LYMPHOHISTIOCYTIC INFILTRATES

CASE NO.	DIAGNOSIS	Leu 1	Leu2A	Leu3A	OKT6	OKT9	OKT10	Leu14/B1	B2	NSE
1	Lymphocytoma	+	+	+	-	++	Nt	+++	+++	++
2	Lymphocytoma	+++ Perifoll	+	+++	++ Perifoll	++ L.F.	Nt	+++	++	++
3	Lymphocytoma	++	+	++	±	Nt	Nt	+++	+++	Nt
4	Lymphomatoid PAP (B)	+++	+	+++	+	-	Nt	-	-	++
5	Lymphomatoid PAP (A)	++++	+++ (++)	+ (++)	±	-	-	-	-	+++
6	Lymphomatoid PAP (A)	+++	+	+++	+ to +++	+++	-	-	-	+++
7	Nodal Lymphoma	-	-	-	-	++++	Nt	-	-	-
8	Jessner's Infiltrate	++++	+	++++	-	Nt	+	-	-	Nt
9	Sarcoidosis	++	+	+++	-	++	Nt	-	-	+++
10	Histiocytosis X	-	-	++	++++	+	-	-	-	++
11	Histiocytosis X	-	-	++	++++	+	-	-	-	++
12	Histiocytosis X	Nt	Nt	Nt	++++	Nt	Nt	Nt	Nt	++
13	CLL	+++	+	++		Nt	Nt	Nt	Nt	Nt
14	MML	-	-	-	-	+++	Nt	-	-	-

CASE NO.	DIAGNOSIS	HLADR	R423	M1	M3	HNK1
1	Lymphocytoma	+++	++	+	+	-
2	Lymphocytoma	Nt	+++	Nt	++ L.F.	+
3	Lymphocytoma	++	+	+	++	Nt
4	Lymphomatoid PAP (B)	+	-	++	+	+
5	Lymphomatoid PAP (A)	+++	-	Nt	Nt	+
6	Lymphomatoid PAP (A)	+++	-	+	+	+
7	Nodal Lymphoma	++++	-	+	-	-
8	Jessner's Infiltrate	+	-	Nt	Nt	-
9	Sarcoidosis	Nt	-	-	+++	-
10	Histiocytosis X	+++	-	-	-	-
11	Histiocytosis X	+++	-	-	-	-
12	Histiocytosis X	Nt	Nt	-	-	-
13	CLL	Nt	-	-	-	Nt
14	MML	Nt	-	++++	+	-

Note: a) Staining pattern in case 5 at 8 days is shown in parentheses ().
b) Staining pattern in Histiocytosis X refers to cells with Histiocytic morphology only.

Key:
++++ = positive staining in ≥ 75% of cells
+++= positive staining in 50-75% of cells
++= positive staining of 25-50% of cells
+ = positive staining of 0-25% of cells
± = equivocal staining pattern
Nt = not tested
Perifoll = staining pattern in perifollicular region
L.F. = staining pattern in lymphoid follicles

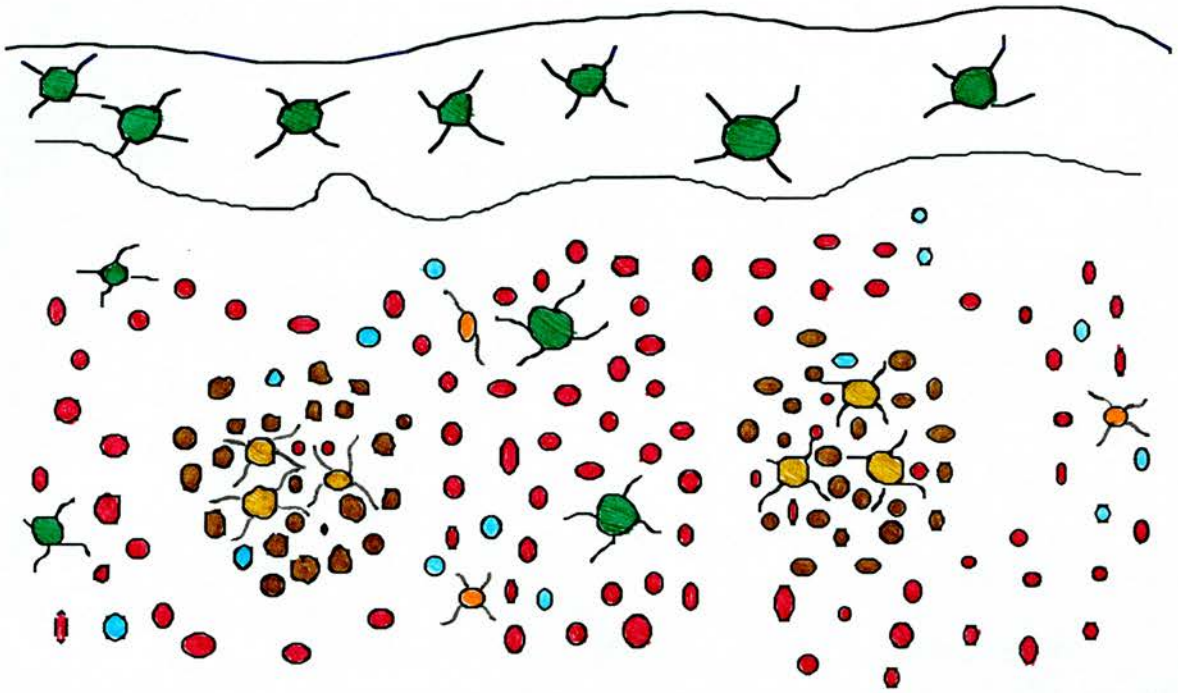


FIGURE 8. **Lymphocytoma Cutis.**
Schematic Representation Of Immunophenotypic Pattern

KEY

- Epidermal OKT6 Dendritic Population (Presumptive Normal Residents)
- OKT6 + Interfollicular Cells
- B Cell. B1 +; Variable B2 Positive
- Follicular Dendritic Macrophage (R423 + LeuM3 +Esterase+)
- T Helper. (Leu3A+)
- T Cytotoxic Suppressor (Leu2A+)
- LeuM1+ Interfollicular Cell

(Sanchez, et al., 1981) drugs (Bernstein, et al., 1974);(Adams, 1981), insect bites (Allen, 1949) and tattoos (Zinberg, et al., 1982). The immunoarchitectural pattern demonstrated here certainly indicates that a dermal lymph node or tonsil equivalent may form under the correct stimulus. The presence of significant numbers of B cells with similar Kappa/Lambda ratios to those observed here, and follicular dendritic cells has been shown in one other study involving cases which showed a diffuse pattern of infiltration (Ralfkiaer, et al., 1984b) and one study involving follicular and diffuse infiltrates (Wirt, et al., 1985).

The mechanism of LF and germinal centre formation may be similar to that in normal lymphoid tissue. In this respect the presence of T cells and follicular dendritic cells is of interest as current evidence indicates that both these cell types (Klaus, et al., 1980) play a key role in GC formation. The follicular dendritic cell appears to be an uncommon visitor to the cutaneous microenvironment as testing of a variety of cutaneous lymphohistiocytic infiltrates (Table 14) shows that R423+ dendritic cells (unlike lymph node or tonsil) are rarely found in skin except in the presence of B cell infiltrates. This observation fits with their role in antigen trapping (Mandel, et al., 1980) when B memory cells are being generated. Experiments in germ free mice also indicate that antigen is normally required for GC formation (Hanna, et al., 1969). Studies of antigen-antibody complexes and C3 receptors similar to those performed in follicular lymphoma (Stein, et al., 1982) are required to further examine the similarities between the GC's in lymphocytoma and normal GC's. This would confirm involvement of an antigen dependent process in lymphocytoma as the etiologies outlined above would indicate.

The origin of the FDC is unknown (Humphrey, 1984) and

TABLE 14-REACTIVITY OF BENIGN AND MALIGNANT CUTANEOUS LYMPHOCYTIC INFILTRATES AND LYMPHOID TISSUE WITH FOLLICULAR DENDRITIC CELL MONOCLONAL ANTIBODY R423. (82 CASES)

HISTOLOGIC DIAGNOSIS	NO. OF CASES TESTED	NO. POSITIVE	PHENOTYPE OF SURROUNDING LYMPHOID CELLS
Tonsillar Lymphoid Follicle	15	15	B
Tonsillar Interfollicular Area	15	0	T
Tonsillar Crypt Epithelium	15	0	T (Exocytic cells)
Lichen Planus	2	0	T
Allergic Contact Dermatitis	8 (13 positive patch test)	0	T
Large Plaque Parapsoriasis	8	0	T
Thymus	2	0	T
Mycosis Fungoides	14	0	T
Sezary Syndrome	1	0	T
Myelomonocytic Leukaemia	1	0	Monocytic
Monocytic Leukaemia	1	0	Not tested
Chronic Lymphocytic Leukaemia	1	0	T
Sarcoidosis	1	0	Macrophage
Eczema	4	0	T cell
Histiocytosis X	2	0	Langerhans' cell
Cutaneous NHL	5	0	T
Cutaneous NHL	3	1	B
Cutaneous NHL	2	0	U
Lymphocytoma Cutis	3	3	B
Lymphocytic Infiltrate of Jessner	1	0	T
Normal Skin (Scalp)	5	0	No infiltrates
Lymphomatoid Papulosis	3	0	T

its migratory capability has previously been debated (Mandel, et al., 1980). The presence of R423 + FDC in lymphocytoma (this chapter) and cutaneous follicular lymphoma (Chapter 6) provides strong evidence that this cell does have the capacity to migrate into extranodal sites and fits with observations of a circulating precursor of the FDC detected by monoclonal antibody KiM4 (Reichert et al., 1983). This cell may, therefore, play a fundamental role in the initiation of the cutaneous infiltrates in this disorder. Germinal centre B cells in contrast lack homing receptors (Reichert, et al., 1983) necessary for the normal recirculation of lymphocytes between blood and/or lymph and tissues (Ford, 1979). The FDC may, therefore, facilitate the establishment of GC B cells in skin.

The B2 antigen has recently been shown to be the C3d receptor (Gerdes and Stein, 1982) and this receptor is strongly expressed by FDC. (Ida, et al., 1983) The B2+ cells observed may, therefore consist of a mixture of FDC and B cells.

The Kappa/Lambda ratio of 4:1 in 1 case would be regarded as monoclonal by at least one group of investigators with considerable experience in this area (Chapter 6). This is pertinent as cases diagnosed as lymphocytoma cutis may eventuate in lymphoma (Shelley and Wood, 1976).

The absence of HNK1+ lymphocytes in 1/2 cases tested is interesting. This contrasts with the uniform presence of HNK1+ lymphocytes in the lymphoid follicles of tonsil (Chapter 4).

2. LYMPHOMATOID PAPULOSIS

The phenotype of Case 1 is similar to that described in other cases of lymphomatoid papulosis type B (Burg, et al., 1981; Willemze, et al., 1983b) and is in agreement with previous labellings of this disorder as a T cell pseudolymphoma (Burg, et al., 1981).

This subgroup is believed to be related to CTCL (Willemze, et al., 1982b) and the Leu1+, Leu3A+ phenotype is certainly similar. However, unlike many cases of CTCL (Chapter 5) significant numbers of OKT9 reactive cells are not observed in this case. OKT9 reactivity has been observed in a few cases of lymphomatoid papulosis in other studies (Ralfkiaer, et al., 1985c). When making comparisons with CTCL it should be mentioned that the division of Ly. pap. into subgroups A and B (Willemze, et al., 1982b) is relatively recent and that although it is logical to assume that CTCL development would develop from cases of type B with their cerebriform morphology rather than subgroup A with its preponderance of Reed-Sternberg like cells prospective or retrospective studies substantiating this are awaited.

The immunochemical pattern found in Case 2 suggests a histiocytic origin for the LAC, including RS like cells, and is in agreement with other studies of Ly. pap. type A (Black, et al., 1972; Beckstead, et al., 1982; Tokura, et al., 1986). Willemze has suggested this pattern is similar to that of indeterminate cells or interdigitating reticulum cells (Willemze, et al., 1983b). A similar pattern has been observed in Hodgkin's disease (Kadin, et al., 1985). The cytochemical similarity between the Reed Sternberg cells of Hodgkin's disease and interdigitating reticulum cells has also been emphasized (Kadin, et al., 1985). One recent study with McAb BerH2 (Cerroni, et al., 1989) also indicates phenotypic differences between the LAC in Ly.Pap types A and B and similarity between the LAC in Ly.Pap type A and Reed Sternberg cells of Hodgkin's disease.

The presence of OKT6+ cells in Ly. Pap type B and their relative absence in type A has been noted previously (Willemze, et al., 1983b). This association is interesting in view of the proven T cell nature of type B lesions and more dubious nature of the LACs in type A.

Until larger numbers of cases of Ly. Pap. type A are studied and a wider range of T cell reagents used the question of the lineage of the LAC in type A lesions remains open. Other studies using different markers have suggested that a proportion of the LAC in Ly. Pap. type A are in fact Leu1- T cells exhibiting aberrant differentiation (Ralfkiaer, et al., 1985). This finding is not inconsistent with their R-S like morphology as T cells undergoing blastic transformation in vitro may develop morphologic features similar to R-S cells. In addition, R-S cells in Hodgkin's disease may occasionally express T cell markers (Kadin, 1985).

The findings in case 3 are of particular interest because of the unusual phenotypic pattern present in the cutaneous infiltrates and the subsequent development of nodal lymphoma. The presence of significant numbers of OKT6+ cells (Plate 59) is atypical of Ly. Pap. type A (Willemze, et al., 1983b). The presence of clusters of cells reacting only with OKT9 (in early biopsies) and ultimately of OKT9 reactivity alone in 80% of the cutaneous infiltrates is similar to patterns observed in cutaneous lymphoma (Chapters 6, 7).

Although the clinical course and cutaneous pathology are both typical of Ly. Pap. type A (history and slides reviewed by Dr. Willemze) one other experienced pathologist (Dr. Costan Berard) considered the cutaneous lesions to be those of malignant lymphoma, mixed lymphocytic - histiocytic (Rappaport) or diffuse, mixed small and large cell type (working formulation). This emphasises the clinico-pathological nature of the diagnosis of Ly. Pap. and difficulty in predicting malignant behaviour in a disease which is often histologically "malignant". Irrespective of the "true" histologic diagnosis, the final outcome and phenotype results suggest that some cases of lymphomatoid papulosis eventually exhibiting malignant behaviour may

demonstrate phenotypes characteristic of lymphoma ab initio.

The nodal histology and immunology are also worth discussing. Five expert lymphoma pathologists in Europe and the United States were asked for their opinion on the lymph node pathology of this patient. The diagnoses given were Hodgkin's disease mixed cellularity with focal lymphocyte depletion (1 European, 3 United States) and malignant lymphoma, large cell immunoblastic polymorphous type (Working formulation)/diffuse histiocytic type (Rappaport) (1 United States). The immunophenotyping demonstrates expression of certain T cell markers on the tumour (Leu4/5) but absence of Leu1 which is normally a pan T marker. The results, therefore, indicate the tumour is of T cell lineage but that it demonstrates aberrant differentiation.

It has recently been suggested that some cases diagnosed as Hodgkin's disease developing in lymphomatoid papulosis are really T immunoblastic sarcomas (Wood, et al., 1986). In terms of basic biology, these differences of opinion may be more apparent than real since 1) activated T cells may develop morphologic features of RS cells (Kadin, 1985). 2) RS like LAC in Ly. Pap. type A may express T cell markers (Wood, et al., 1986). 3) RS cells in Hodgkin's disease may show weak expression of T cell markers (Abdulaziz et al., 1984; Kadin, et al., 1988). 4) This case, in which there was divided opinion between Hodgkin's disease and immunoblastic sarcoma displayed reactivity with some T cell monoclonals.

The relationship between the neoplastic cells of Hodgkin's disease and T cells is further strengthened by a) variants of MF which show morphologic features of Hodgkin's disease (VanDerPutte, et al., 1982b), b) the histologic spectrum of Ly. Pap. with some cases eventuating in MF (Fine, et al., 1974) or Hodgkin's disease (Kadin, 1985) and c) the simultaneous occurrence of MF and Hodgkin's disease in the same patient (Hawkins, et al., 1983). In addition

(although this does not prove their lineage or origin) cells with RS morphology have been identified in human thymus (Thomson, 1955).

It has been suggested previously that Reed Sternberg cells were derived from follicular dendritic cells (Curran and Jones, 1978). However, their lack of reactivity with R423 (Abdulaziz, et al., 1984), and the negative reaction obtained with R423 here in RS like cells in Ly. Pap. type A and one malignancy developing from it fail to support this contention.

The presence of LeuM1 positivity in a small proportion of infiltrating cells in the malignant lymph node is consistent with Reed Sternberg cells (Hsu, et al., 1986) or a T cell derived tumour (Hanjan, et al., 1982).

The immunologic diversity of the 3 cases of lymphomatoid papulosis examined is consistent with the postulate that the histologic diagnosis of lymphomatoid papulosis encompasses more than one disease (Brehmer-Andersson, 1981). The disparate histologic diagnoses by experts in lymph node and skin in case 3 suggests further immunophenotypic studies are required to more fully characterize nodal and cutaneous disorders involving Reed Sternberg or Reed Sternberg-like cells.

3. LYMPHOCYTIC INFILTRATE OF JESSNER

The relationship between LIJ and lymphocytoma has been debated in the past.

Some authors (Postma and Sluiter, 1958) consider LIJ to be a variant of lymphocytoma. Whereas, others (Calnan, 1957; Wolf, 1957; Lange, et al., 1982) consider them distinct clinicopathological entities. The B cell nature of lymphocytoma has already been discussed. Burg considered LIJ also to be a B cell pseudolymphoma (Burg, et al., 1982), although corroborative immunologic studies were not done. However, studies utilizing cytochemistry (Konttinen, et al., 1981) and heteroantisera with immunofluorescence (David, et

al., 1980) indicate that the majority of infiltrating cells in LIJ are in fact T cells.

The results obtained here indicate that the infiltrate tested consists mainly of T cells. This is in agreement with 2 other studies (Willemze, et al., 1984; Ralfkiaer, et al., 1984b) utilizing McAbs, and as such contrasts with the findings in lymphocytoma. The H/S ratio in the case tested is 4:1, similar to those found in the study of Willemze, et al. (Willemze, et al., 1984) (2:1 to 6:1) whereas suppressor cells were preponderant (H/S 1:2) in the case reported by Ralfkiaer (Ralfkiaer, et al., 1984b).

Although a mature T cell infiltrate is identified, dermal OKT6+ dendritic cells are absent. A similar phenomenon has been noted by Willemze et al. (Willemze, et al., 1984) This situation contrasts with the normal Leu1+ interspersed OKT6+ pattern found in most T cell infiltrates (Chapters 4, 5, 6). Analysis of additional cases of LIJ is required to determine whether this is the usual pattern observed in this condition.

4. SARCOIDOSIS

A predominance of esterase positive epithelioid histiocytes has been shown in one other recent study of sarcoid (Konttinen, et al., 1983). Transferrin receptor expression (OKT9 positivity) has been shown in one other non-malignant histiocytic disorder, sinus histiocytosis (Vandenoord, et al., 1985) and in this respect the sarcoid histiocyte is similar to the starry sky or tingible body macrophage of tonsil and lymph node (chapter 4). The latter cell is also esterase and probably LeuM3 positive (chapter 4).

A similar T cell pattern with an excess of Leu3A+ helper T cells and peripheral distribution of Leu2A+ suppressor T cells has been observed elsewhere (Modlin, et al., 1983) and apparently is typical of sarcoidosis, tuberculosis and tuberculoid leprosy whereas in lepromatous

leprosy and rhinoscleroma the Leu2A (OKT8+) mantle is absent (Modlin, et al., 1983).

Mishra (Mishra, et al., 1983) using different B cell (T015) and Langerhans' cell/cortical thymocyte (NA134) reagents found a similar dearth of B cells and Langerhans' cells in sarcoidosis.

In keeping with routine histopathology exocytic Leu1+ cells are absent in contrast to biopsies of KVEIM and PPD reactions where OKT3+, Leu1+ T cells may be seen in the epidermis (Mishra, et al., 1983).

T cells have previously been demonstrated in sarcoidosis with immunoperoxidase and a heteroantiserum (anti-HTLA) (Chu, et al., 1979). Bronchial washing studies (Hunninghake and Crystal, 1981) have shown an excess of helper T cells (without architectural information).

Without the benefit of functional studies, one can only speculate as to the interactions between the T cells and epithelioid histiocytes in this disease. Experiments in thymectomized mice injected by M. Leprae (Tanaka and Emori, 1980) and S. Mansoni (Colley, 1981) indicate that T cells augment and accelerate the epithelioid granulomatous response (Rothwell and Spector, 1972).

Although an infectious etiology has never been proven in sarcoidosis the immunoarchitectural similarity between this disease and other infectious diseases with active host response (tuberculosis and tuberculoid leprosy) is striking.

5. HISTIOCYTOSIS X

The OKT6+, HLADR+ phenotype observed confirms recent observations (Harrist, et al., 1983b) and enhances the concept that HX cells are of the Langerhans' cell lineage. Leu3A positivity of HX cells is similar to findings with OKT4, (Harrist, et al., 1983b). Until recently it was thought that Leu3A resided solely on helper T lymphocytes. It has since been shown that macrophages and occasional normal Langerhans' cells can express the Leu3A/OKT4

determinants (Wood, et al., 1983a; Moscicki, et al., 1983) Leu3A reactivity on Langerhans' cells may, therefore, be due to activation from a variety of causes and not synonymous with neoplasia.

The OKT4/Leu3A molecules appear to be involved in T cell recognition of Class II HLA antigens (Meuer, et al., 1984) promoting cell adhesion between T cells and antigen presenting cells (APC). The demonstration here and elsewhere (Harrist, et al., 1983b) of Leu3A+/OKT4+ reactivity in HX cells (normally HLADR+) and of HLADR on activated T cells (Evans, et al., 1978) (Leu3A+, OKT4+ on the helper subset) suggests these molecules may be involved in T cell APC interaction and might even be transferred between the two cell lineages.

The role of Leu3A/OKT4 in T cell proliferation (Biddison, et al., 1983) raises the possibility that these molecules might also be directly involved in an amplification of Langerhans' cells during delayed type hypersensitivity reactions. The easier demonstration of Leu3a/OKT4 in malignant Langerhans' cells compared to normal Langerhans' cells would then imply that the Leu3A/OKT4 molecules are somehow directly involved in driving the neoplastic growth of Langerhans' cells observed in HX. This, of course, is merely supposition and would require further experimental testing.

The number of histiocytic cells expressing the Leu3A marker in our cases is smaller than that observed by Harrist et al. (Harrist, et al., 1983b) and may reflect a difference in sensitivity of laboratory techniques used or a true heterogeneity among HX cases in the expression of Leu3A.

The reactions with the other monoclonals indicates that an admixture of T cells is present, a larger number being found in Case 2 which on routine light microscopy had a more mixed infiltrate than Case 1. The My11 positivity in Case 2 is probably due to reactivity of this antibody with E

rosette positive (T) lymphocytes. At least some of the Leu1+, OKT3+ lymphocytes in Case 2 are phenotypically suppressor (Leu2A+) cells. The functional role of these and the HNK1+ cells is open to speculation. It is interesting that Leu2A+ suppressor cells and HNK1+ (killer/natural killer) cells are not identified in Case 1 which had a more aggressive course. Since the study was initiated Case 1 has died despite Interferon therapy.

The varying numbers of lymphocytes (eg., Leu2A+, HNK1+) found in the cases examined suggests that future studies should be performed to assess whether their presence or absence bears any meaningful relationship to prognosis.

The results in this study and elsewhere suggest that an OKT6+ phenotype will be characteristic of many cases of HX. The HX phenotype, therefore, contrasts with that found in large cell (or "Histiocytic") lymphomas (Chapter 6). Although OKT6+ cells may be identified in the cutaneous infiltrates of many cutaneous diseases including lymphomas (Chapters 5, 6), the dearth of cutaneous histiocytic disorders with a preponderantly OKT6+ phenotype suggests the OKT6 marker may have potential use as an adjunct diagnostic aid in HX. However, clinicopathological data would probably be required in addition as other pediatric disorders may involve proliferation of Langerhans' cells; eg., Hashimoto-Pritzker Histiocytosis (Hashimoto and Pritzker, 1973).

The OKT6+ subpopulation normally forms an insignificant fraction of circulating mononuclear cells (Kung, et al., 1980). Peripheral blood studies were not performed in this study. The OKT6+ phenotype demonstrated here and elsewhere suggests that detection and quantification of OKT6+ cells in peripheral blood in HX would confirm leukaemic dissemination and could be correlated with multiple organ involvement and requirement for early chemotherapy.

6. CHRONIC LYMPHOCYTIC LEUKAEMIA

The Leu1+ phenotype demonstrated is compatible with B

or T CLL (Schroff, et al., 1982). However, the Leu2A and/or Leu3A positivity seen in the case examined has not been recorded in B CLL (Schroff et al., 1982) the more common type (normally B1+, B2+/-, Leu1+, Leu2A-, Leu3A-)

whereas, T CLL, which commonly involves skin (Sumiya, et al., 1973; Lillie, et al., 1973), is usually positive for Leu3A, Leu2A or both (B1-, B2-, Leu1+, Leu2A+/Leu3A+) (Schroff, et al., 1982). The reaction with subset antibodies Leu2A/Leu3A, therefore, suggests this case belongs to subset 1 of T CLL (Schroff, et al., 1982) (Leu3A+/Leu2A+).

The lack of OKT6 reactivity in overlying epidermis is remarkable as this is the only specimen of the benign and malignant infiltrates examined in this project with an OKT6- epidermis. As other LC markers were not employed and non-infiltrated skin was not examined in this patient, the significance of this finding is unknown.

7. MYELOMONOCYTIC LEUKAEMIA (MML)

To date there has been a lack of other studies of MML using similar McAbs. The LeuM1+, Leu1-, Leu2A-, Leu3A-, OKT6-, B1- phenotype of the infiltrating cells contrasts with T cell ALL (Schroff, et al., 1982) (LeuM1-, Leu1+, Leu3A+/-, LEU2A+/-); pre B/common ALL (Schroff, et al., 1982) (LeuM1-, B1+/-); adult T cell leukaemia (Nakahara, et al., 1982) (LeuM1-, Leu1+. Leu3A+); B CLL (Schroff, et al., 1982) (LeuM1-, B1+, B2+/-, Leu1+, Leu2A-, Leu3A-); and T CLL (Schroff, et al., 1982) (LeuM1-. B1-, B2-, Leu1+, Leu2A+/-, Leu3A+/-)The OKT6- phenotype contrasts with that of Histiocytosis X.

Although the myeloid McAbs (table 2) were not available when this case was tested, myeloid/monocyte monoclonals have been used to further categorize the monocytic and non-monocytic variants of AML (Sondel, et al., 1981; Nakahara, et al., 1982).

The esterase negativity of the MML infiltrate contrasts with sarcoidosis (NSE+) and also contrasts with pure monocytic leukaemia (Schilling type) which demonstrates strong esterase positivity (Shaw and Nordquist, 1975). Hayhoe emphasises the difficulties in precisely categorizing monocytic leukaemias according to their presumed cell of origin (Hayhoe, 1960). In this sense,this case is interesting as naphthol A.S.D Chloracetate Esterase staining (granulocytic marker) was also negative.

Schiffer (Schiffer, et al., 1975) suggested on the basis of skin window studies that cutaneous monocytic leukaemia cells may be proliferating in situ as well as demonstrating the capacity of circulating monocytoid cells to migrate into tissues. The OKT9 reactivity (proliferation associated antigen) supports this theory.

The LeuM1 antigen is readily demonstrated on circulating monocytes with relatively few LeuM1+ cells in non-malignant lymphoid tissue. (Chapter 4) The LeuM3 antigen is readily demonstrated in lymphoid tissue macrophages (Chapter 4). The finding of LeuM3 positivity in a proportion of infiltrating leukaemic cells raises further questions concerning expression of LeuM1/LeuM3 on the normal monocyte as it circulates and finally emigrates into tissues.

It is unknown whether the differences observed between sarcoidosis (NSE+, LeuM3+, LeuM1-, OKT6-), myelomonocytic leukaemia (NSE-, LeuM1+, LeuM3-, OKT6-) and Histiocytosis X (NSE+/-, LeuM3-, LeuM1-, OKT6+) likewise reflect origins in different subsets of the monocyte/macrophage series or variable expression of markers during the life cycle of the normal monocyte i.e. LeuM3+ in tissues and LeuM1+ in blood.

This particular case shows no evidence of co-expression of lymphoid and myeloid markers as described by Scamurra et al. (Scamurra, et al., 1983)

CHAPTER EIGHT

DISCUSSION, SUMMARY, AND CONCLUSIONS

Including:

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CHAPTER EIGHT

DISCUSSION, SUMMARY, AND CONCLUSIONS

I. DISCUSSION

Prior to 1980 the techniques used to establish the cellular identity of lymphomas were limited to rosetting techniques, immunofluorescence or immunoperoxidase in conjunction with heteroantisera, and enzyme cytochemical techniques (chapter 2). Application of heteroantisera on cell suspensions and in situ did permit categorization of lymphomas into T and B types. T cell lymphomas could also be roughly categorized into early and late thymic types (Chapter 2). The limitations of these methods have been discussed in Chapter 2.

In the introduction an idealized method of tissue identification of lymphoreticular cells was outlined(Table 1, McMillan, 1985). The advent of monoclonal antibodies directed against lymphoreticular subpopulations has permitted us to come closer to this ideal. Despite initial technical difficulties caused by the effect of fixatives on the antigens involved (Chapter 3) (McMillan, et al., 1981a,b,c; 1982a) in situ studies utilizing McAbs have proved to be practicable. The technique is simple and rapid. The expense of some of the early commercially available reagents should improve as more of these become available. McAbs directed against differentiation antigens have been produced and the distribution of the appropriate

determinants in non-neoplastic tissue (thymus, tonsil, lymph node) has been outlined (Chapter 4). However, the studies of extrathymic lymphoid tissue (Chapter 4) and cutaneous lymphocytic infiltrates (Chapters 5, 6, 7) indicate that these antigens of "immaturity" are not confined to intrathymic cells undergoing normal development. For instance, OKT9+/OKT10+ cells are readily found in tonsil (Chapter 4) and OKT9+ cells are occasionally found in non-malignant cutaneous lymphocytic infiltrates (Chapter 5).

Although the McAbs provide improved specificity over heteroantisera, the determinant itself may not be confined to a particular subtype of cells. This point is exemplified by the findings of OKT6 which, when initially used, was thought to be specific for "common" thymocytes at an intermediate stage of thymic differentiation, but was subsequently found to react with a dendritic population in epidermis (Chapter 4), tonsillar crypt epithelium and lymph node (Chapter 4), and with cells in a variety of cutaneous lymphocytic infiltrates (Chapters 4, 5, 6, 7).

Preliminary studies have also been done on paraffin sections (Andrade, et al., 1988; Cerroni, et al., 1989). These are mainly limited to the broad categorization of infiltrates into T and B types as the range of antigens detected by McAbs reacting with paraffin embedded tissue sections is presently narrow. An additional hindrance is lack of specificity for distinct cell types eg. sharing of

the respective antigens by T and B cells. However the use of formalin fixed paraffin sections often produces histologic sections with excellent preservation of tissue architecture.

The technique is also applicable to ultrastructural studies as shown by several groups (Murphy, et al., 1981; Holden et al., 1982c). The authour's efforts in this direction (unpublished observations) do, however, indicate that some of the antigens detected by McAbs (eg., OKT3) may be more sensitive to EM processing techniques than antigens detected by heteroantisera.

The technique provides information on in vivo relationships as shown in the topographic studies of tonsillar tissue (Chapter 4) and in the Pautrier microabscesses in MF (Chapter 5) (Plate 34).

Although most antibodies tested produce interpretable results on tissue section, the variable strength of staining within a cell population and day to day variations in the technique suggest that further improvements in technology are desirable. One such improvement is the Avidin-Biotin technique (Hsu, et al., 1981) which appears to produce more consistent results. Many of the reagents are now widely available, eg., OKT and Leu series, and this will permit similar testing in numerous laboratories. In addition, double labelling may even be done with the immunoperoxidase technique (Mackie, and Turbitt, 1982; Ralfkiaer, et al.,

1985a). The variation in intensity of staining throughout cellular infiltrates and presence of dense populations in some cases often makes accurate quantification difficult and the technique is semiquantitative at best. However, the preponderant phenotype of a cell population can usually be readily identified and this feature is useful in lymphoma categorization. In addition, minority markers, eg. HNK1, can usually be easily identified (Chapters 5, 6). In this sense the new technology satisfies some of the frustrations encountered by the author one decade previously and permits a more revealing dissection of lymphoma into cells of varying differentiation, and putative immune reactive cells.

One of the histologic criteria for malignancy is "dedifferentiation". It is uncertain how closely this resembles the less differentiated state of the maturing healthy cell.

The differentiation disturbances discussed in Chapter 2 could have immunopathologic corollaries relevant to diagnosis:

1) Failure or block of differentiation would result in a phenotype similar to one of the earlier stages of T cell (Table 5) or B cell (Table 10) ontogeny. This would produce a) an accumulation of cells expressing immature determinants (eg. OKT9, OKT10) or double markers (eg. OKT4/8, Leu3A/2A) b) a deficiency or "loss" of all mature surface markers found on peripheral lymphocytes (eg. OKT3, T11, Leu1).

2) A stem cell disorder with clonal expansion and preponderance of differentiated compartments would be similar to benign proliferations when examined with mature markers (mature marker deficiency would not be evident). Subset markers (eg. Kappa/Lambda, helper/suppressor) might reveal clonal expansion although clinical utility of this feature would depend on the degree of overlap with reactive states. The increased proportion of dividing stem cells might be evinced by proliferation associated markers eg. transferrin receptor, (5E9, OKT9). Again comparison would have to be made with benign disorders. Degrees of phenotypic overlap with benign infiltrates would be expected when the proliferative fraction is relatively low.

Since certain markers may indicate immaturity and/or proliferation eg. OKT9 it may be difficult to distinguish category 1 and 2 unless mature surface marker deficiency is present.

3) Aberrant differentiation could produce a) selective loss of markers characteristic of a certain stage of differentiation with retention of others (eg. OKT3 and T11) resulting in a phenotype not recognized during normal development (aberrant phenotype). When larger batteries of McAbs are used cases initially grouped as category 1)b) might, however, require recategorization into this group. This category must remain tentative. It's presence would imply a more profound qualitative abnormality in malignancy

than categories 1) and 2) which would present mainly with quantitative phenotypic abnormalities, eg. increased fraction of proliferating cells. Tumours expressing aberrant phenotypes may in fact represent the expansion of hitherto unrecognized T or B subpopulations. These could be revealed by more extensive studies of thymus and B cell zones of non malignant lymphoid tissue. This category is, therefore fascinating as it touches on possible fundamental differences between benign and malignant lymphocytes.

b) putative tumour specific markers. This groups unique feature would be a determinant confined to malignant cells. This would differ from 3a) where individual markers found on normal cells are combined in a pattern peculiar to malignancy.

Although the phenotypic studies permit further speculation on the pathogenesis of certain disorders, eg. CTCL (Chapter 5), their practical diagnostic and therapeutic use also needs consideration. This will now be outlined, and the results of other laboratories will also be discussed as a large number of groups have been involved in this area during the time course of this project. Recent alternative methods of lymphoma diagnosis will also be discussed.

A. DIAGNOSIS OF LYMPHOMA BY IMMUNOPHENOTYPING

SKIN

Attempts at the diagnosis of CTCL and CBCL using monoclonal antibodies have concentrated on three areas:

1) an imbalance in the ratio of markers expressed by normal lymphocytes; 2) the presence of so-called immature determinants and 3) the possible existence of antigens expressed solely by malignant cells.

1) IMBALANCE IN RATIOS OF MARKERS EXPRESSED ON NORMAL LYMPHOCYTES

a) HELPER/SUPPRESSOR RATIO AND DOUBLE MARKER EXPRESSION IN CUTANEOUS T CELL LYMPHOMA

McAb studies have demonstrated that CTCL is phenotypically of the mature helper subset in most cases (reviewed in Chapter 5). This situation, therefore, contrasts with T cell ALL (Reinherz, et al., 1980) and lymphoblastic lymphoma where the lymphocytes are phenotypically "early" or "common" (cortical) thymocytes (Bernard, et al., 1981) indicating a block in differentiation. (See Thymus, Chapter 4) As discussed in Chapter 5 this may reflect the presence of a stem cell disorder in MF with resultant amplification of all compartments, the more differentiated ones remaining larger as in benign lymphoid populations. The mature surface marker profile would then be similar to normal lymphocytes in the T cell zones of lymphoid tissue (helper T, Chapter 4) or reactive lymphocytic infiltrates (helper T, Chapters 4 and 5). Phenotypic abnormalities might only then be detected by examining the cell population in question with proliferation associated markers (eg. transferrin receptor) or if other forms of disturbed differentiation are present

eg. differentiation block or aberrant differentiation (see below and results in MF, Chapter 5). The demonstration that a malignant infiltrate is "helper" may, therefore, be useful confirmatory evidence of a diagnosis of CTCL. There is, however, considerable variation in the "suppressor" content in CTCL lesions (Chapter 5) and rare cases may have an OKT8 positive phenotype. The varying proportions of "helper" and "suppressor" cells found in CTCL lesions has been reviewed (Chu, 1983).

Whether McAbs against lymphocyte subsets may be used to differentiate malignant from benign infiltrates is uncertain. The overlap between benign and malignant conditions make this unlikely. A preponderantly "helper" T cell infiltrate is also present in 1) lichen planus (Bhan, et al., 1981b; McMillan, et al., 1981a; Gomes, et al., 1982; DePanfilis, et al., 1983) (Chapter 6), 2) atopic dermatitis (Zachary, et al., 1984) (Chapter 6), 3) lymphomatoid papulosis (Burg, et al., 1981; Harrist, et al., 1981; Willemze, et_al., 1983 b) (Chapter 8), 4) chronic eczematous dermatitis of non-specified type (Chapter 6), 5) LPAP (McMillan, et al., 1982f; Olsen, et al., 1982) (Chapter 6)psoriasis (Baker, et al., 1984), 7) lymphomatoid granulomatosis (Harrist, et al., 1981) and 8) allergic contact dermatitis (McMillan et al., 1983c; Scheynius, et al., 1983) (Chapter 4). The presence of a helper T cell infiltrate per se, therefore, is not synonymous with CTCL.

Clinicopathologic data are mandatory.

Since an OKT8+/Leu2A+ suppressor phenotype is apparently present in actinic reticuloid (Chu, 1983) McAbs against mature subset antigens may be of some use in separating this condition from CTCL.

Monoclonal antibodies against mature T cell markers have been used in conjunction with immuno-electron microscopy to analyze more accurately the lymphoid component of CTCL. (Hashimoto, and Iwahara, 1983) Using this technique, the nuclear convolutions of accompanying histiocytes are not included in the overall morphometric assessment. This approach may yield further useful data when performed with immature markers.

Double marker expression might also be utilized in identifying malignant T cell infiltrates and there is evidence that cells expressing both OKT4 and OKT8 are present in CTCL infiltrates (Chapter 5). Double labelling of mycosis fungoides infiltrates has been performed using immunofluorescence (Chu et al., 1982a) and immunoperoxidase (Mackie and Turbitt, 1982) methods. Whether these methods are sufficiently sensitive to identify malignancy in its early stages remains uncertain. Recently developed immunogold techniques (Holden, and MacDonald, 1983) applied to immunoelectron microscopy should permit electron microscopic double labelling of lymphoid cells and allow for accurate analysis of such cells in benign and malignant

infiltrates.

b) LIGHT AND HEAVY CHAIN RESTRICTION IN CUTANEOUS B CELL LYMPHOMA

Normal and reactive lymphoid tissues contain a heterogeneous population of B lymphocytes (Curran, and Jones, 1977). Immunofluorescent or immunoperoxidase staining shows a mixture of Kappa and Lambda light chain bearing cells and a similarly heterogeneous reaction with antibodies to the various heavy-chain determinants. In contrast, a lymphomatous cell population which represents a preponderantly monoclonal expansion of one cell (Fialkow, 1976) exhibits so-called light and heavy chain restriction. The difference, however, is not absolute (Chapters 6, 7), and the criteria for diagnosing Kappa or Lambda monoclonality do not necessarily imply an autonomous neoplasm, as lymphoproliferative disorders occurring in this context may disappear on withdrawal of immunosuppressive therapy, (cyclosporin and corticosteroids) (Starzl, et al., 1984).

Heteroantisera have been used to demonstrate monoclonality in CBCL (Barr, et al., 1980) and more recently McAbs have been used in a similar manner (Wood, et al., 1983b) (Chapter 6). Further comparative studies of CBCL and lymphocytoma cutis are required.

2) MARKERS OF IMMATURITY (OKT6, NA1/34, BL6, OKT9, 5E9, B3/25, OKT10)

Monoclonal antibodies OKT6 or NA1/34, BL6, OKT9, and OKT10 react mainly with immature cortical or subcapsular thymocytes (Chapter 4). In contrast, medullary thymocytes show a higher density of mature markers detected by McAbs such as OKT3/OKT1 and Leu4/Leu1 (Chapter 4). Studies with these antibodies indicate certain lymphomas do preferentially express antigens found on immature thymocytes during ontogeny. (Chapter 6)

In cutaneous infiltrates the significance of cells reactive with OKT6, NA1/34, (McMillan, et al., 1981b; McMillan, et al., 1982d; Mackie, and Turbitt, 1982; Holden et al., 1982a; Chu, et al., 1982a) BL6 (Schmitt, et al., 1982), OKT9 (Kung, et al., 1981; McMillan, et al., 1982e; Turbitt and Mackie, 1986) and OKT10 (Chu, et al., 1983a; McMillan, et al., 1983b; McMillan, et al., 1984; Turbitt and Mackie 1986) is uncertain since 1) OKT6 and NA1/34 cross react with Langerhans' cells (McMillan et al., 1981b; Fithian, et al., 1981; Chu, et al., 1982b; Dubertret, et al., 1982; Schmitt, et al., 1982; Harrist, et al., 1983a) 2) OKT9 reacts with the transferrin receptor (which is present on a wide range of replicating cell types) (Goding and Burns, 1981; Sutherland, et al., 1981; Trowbridge, et al., 1981) and 3) the OKT10 determinant is present on some activated T cells, bone marrow progenitor cells, (Kung, et al., 1980) plasma cells, (Bhan, et al., 1981a) and K/NK

cells (Herberman, and Ortaldo, 1981). The significance of OKT6/9/10 reactivity has been discussed earlier (Chapter 5). The immunophenotypic profiles of mycosis fungoides (Chapter 5) indicate OKT9 expression often signifies proliferating stem cells. However, in a small proportion of cases with simultaneous deficiency of mature surface markers (phenotypic profile compatible with a differentiation block) OKT9 reactivity may be due to the presence of cells similar to those found in the early stages of thymic differentiation (Table 5).

The preferential expression of OKT9 (Chapter 5), 5E9 (another transferrin antibody) and OKT10 reactivity in CTCL (Chapter 5) is obviously of interest. However, the fact that these antibodies are not restricted to lymphoma cells suggests that diagnostic tests using these markers may be difficult to interpret.

3) LOSS OR DEFICIENCY OF MATURE T CELL MARKERS

Loss of mature T cell markers has also been documented in several McAb studies of CTCL (Kung, et al., 1981; Chu, et al., 1983a; Chu, et al., 1982a; Holden, et al., 1982b; McMillan, et al., 1982c; Schmitt, et al., 1982; Wood, et al., 1982; Willemze, et al., 1983a) (Chapter 5). This phenomenon tends to be most apparent in advanced cases. It is unknown whether this biologic feature can be used regularly as a diagnostic tool. Its presence in only 3 of 26 cases of CTCL (3 of 23 MF) suggests limited application,

at least with the reagents used in this study. Abel, et al., (1985) and Wood et al., (1986) have suggested more universal use of Leu 8 and Leu 9 deficiencies in diagnosing MF. Leu 8/9 deficiency cannot, however, discriminate benign from malignant erythrodermas (Abel, et al., 1988).

4) ABERRANT DIFFERENTIATION

Phenotypes not regarded as part of normal thymic differentiation (Table 5) may occur in mycosis fungoides (Chapter 5) and cutaneous lymphomas of non MF/SS types (Chapter 6). Again these are uncommon in MF (3 of 23 cases) and it remains to be seen whether these would be helpful in predicting the outcome of an atypical lymphocytic infiltrate.

5) "TUMOUR SPECIFIC" MARKERS

The ideal diagnostic McAbs would be specific for lymphoma or individually for CTCL and CBCL. The McAbs Be1 and Be2, which react with CTCL cells (Berger, et al., 1982; Berger and Edelson, 1983) come closest to this on preliminary screening studies. The studies done here, however, show that their expression in malignancy is preferential rather than specific and considerable overlap exists between reactive and malignant states. They also react with normal tissue constituents [Be1, follicular epithelium; Be2, dermal endothelium and tonsillar interfollicular area (Chapter 4)]. In addition, not all lymphomas react with these antibodies. The reactivity of 54

cutaneous lymphohistiocytic infiltrates and non-neoplastic lymphoid tissue with McAbs Be1 and Be2 is shown in Table 15. A similar spectrum of reactivity of Be2 with normal skin, reactive and malignant lymphocytic infiltrates has been reported by one other group (Ralfkiaer, et al., 1986). Be1 was not studied.

The diagnostic usefulness of these reagents in blood is discussed in the next section.

BLOOD

1) T CELL LYMPHOMA LEUKAEMIA

Problems in the interpretation of McAb studies in blood have been previously reviewed (McMillan, 1983).

Studies on circulating peripheral blood lymphocytes (PBL) have not been performed here. However, for sake of completion results of recent studies will be mentioned.

Using conventional surface markers (E, EAC rosetting) the percentage of T cells in peripheral blood may be a significant prognostic parameter (Van Der Loo, et al., 1981) in CTCL, with less than 55 per cent T cells being an unfavourable sign. The diagnostic use of T cell and T subset quantification of PBL in CTCL is unclear.

The phenotype of circulating lymphoid cells in Sezary syndrome and leukaemic CTCL is OKT3/OKT4 positive (Boumsell, et al., 1981; Haynes, et al., 1981; Laroche and Bach, 1981; Thivolet, et al., 1984) but unlike most T cells is 3A1 negative (Haynes, et al., 1981). This phenomenon may be due

TABLE 15-REACTIVITY OF 54 CUTANEOUS LYMPHOHISTIOCYTIC INFILTRATES AND NON-NEOPLASTIC LYMPHOID TISSUE WITH MONOCLONAL ANTIBODIES BE1 AND BE2

Disease	BE1 No. Pos/ No. Tested	BE1 Range of positively staining cells	BE2 No. Pos/ No. Tested	BE2 Range of positively staining cells
CTCL/MF	9/11	0-80%	6/10	0-60%
CTCL/SS (erythrodermic)	3/3	5-60%	3/3	30-70%
Non-CTCL erythroderma	0/3	0%	0/3	0%
Cutaneous lymphoma (non MF/SS type)	5/11	0-70%	6/11	0-80%
Large plaque parapsoriasis (LPAP)	1/5	0-10%	1/5	0-25%
Lymphomatoid papulosis (L. PAP)	1/1	20%	1/1	20%
Atopic dermatitis (AD)	1/5	0-70%	1/5	0-40% (weak staining)
Allergic contact dermatitis (ACD)	1/4	0-10%	0/3	0%
Histiocytosis X	2/2	20-50%	2/2	10-20%
Chronic lymphocytic leukaemia (CLL)	±	±	±	±
Lichen planus	1/3	0-15%	2/3	0-40%
Atypical Lymphocytic Infiltrate	0/2	0%	2/2	5-20%
Granulocytic sarcoma	0/1	0%	0/1	0%
Lymphocytic infiltrate of Jessner	0/1	0%	1/1	20% (weak)
Sarcoidosis	1/1	60%	1/1	50%
Lymph node (reactive)	0/1	0%	0/1	0%
Tonsil	0/2	0%	2/2	5-10% (weak)

Key: ± = Equivocal Reaction

to malignant expansion of a 3A1 negative subpopulation within the lymphoma. This possibility has been made more plausible by the demonstration of a Sezary-like cell having an OKT3+, OKT4+, 3A1- phenotype in normal peripheral blood. This may be the normal counterpart of the neoplastic Sezary lymphocyte (Matutes, et al., 1983). Presumably, double labelling with OKT4 and 3A1 might identify Sezary cells in early cases.

Immuno-EM may also be valuable, since the phenotype of Sezary cells varies with the clinical expression of disease. For example, an OKT8 positive phenotype may be observed in chronic actinic dermatitis (Chu, et al., 1983b).

Nevertheless, the usefulness of the blood helper/suppressor ratio in diagnosing CTCL is uncertain. When erythrodermic patients with and without CTCL were studied a significant difference in the H/S ratio was observed in one study (Willemze, et al., 1983a). In contrast, another report claimed the helper/suppressor ratio to be of little help in differentiating CTCL from benign dermatosis. However, the per cent reactivity of PBL with Be1 and Be2 was claimed to be of diagnostic value (Chu, et al., 1983c). In addition, circulating Be1 and Be2 reactive cells have been detected in patients who were not diagnosed as leukaemic by routine methods. This has been claimed to indicate that CTCL is a disseminated process at a very early stage (Berger, and Edelson, 1983). When one considers the

migratory capacity of normal lymphocytes this proposition appears reasonable. However, the Be1/Be2 positivity of reactive lymphocytes (Chapters 4, 5) indicates that equating Be1/Be2 positivity with clonal neoplastic cells would be premature.

2) B CELL LYMPHOMA

Small numbers of circulating monoclonal B lymphocytes have been described in patients with systemic B cell lymphomas (Ault, 1979). This suggests that similar techniques might be utilized to detect subtle spread in patients with primary CBCL or atypical B cell infiltrates, and lymphocytic infiltrates of uncertain prognosis, eg., lymphocytoma cutis.

B. ALTERNATIVE METHODS OF LYMPHOMA DIAGNOSIS AND CATEGORIZATION

Routine light microscopic diagnosis and categorization of lymphoma is hampered by the morphologic similarities between

1) normal transformed lymphocytes and lymphoma cells, (Chapter 6) and between

2) malignant T and B lymphocytes (Chapter 7).

Electron microscopy (Lutzner, et al., 1971), DNA cytophotometry (Van Vloten, et al., 1974), morphometry (Meijer, et al., 1980), and chromosomal analysis (Whang Peng, et al., 1982) are existing ancillary methods of diagnosis. The first three methods have limitations of

specificity, the latter, sensitivity. Their application is mainly in diagnosis rather than categorization and they are limited to specialized centres.

GENE REARRANGEMENT TECHNIQUES

Recently, the rearrangement of T cell receptor (TCR) genes and immunoglobulin heavy- and light-chain genes have been shown to be highly sensitive markers of clonality and lineage of non-Hodgkin's lymphomas (Arnold, et al., 1982; Flug, et al., 1985). The sensitivity of DNA hybridization permits the detection of clonality in 1% of the total volume of tissue sampled (Cleary, et al., 1984). Current areas requiring clarification with this approach will be outlined.

LINEAGE ASSIGNMENT

Rearrangement of immunoglobulin heavy-chain genes is not specific for B cell neoplasia and may be seen in myeloid and T cell leukaemias (Flug, et al., 1985; Kitchingman, et al., 1985). In a similar manner rearrangement of the T cell receptor gene may be seen in B cell lymphomas and leukaemias (Pelicci, et al., 1985).

Rearrangement of the light-chain immunoglobulin gene is regarded as much more specific for B cell lineage than rearrangement of heavy-chain genes. However, rarely, light-chain rearrangement has been found in lymphomas showing simultaneous TCR BETA chain gene rearrangement and T cell immunophenotype (Hakawa, et al., 1987; Sheibani, et al., 1987). These discrepancies may be explained by chromosomal

breakage producing an altered light chain band, or they may be due to the presence of separate clonal populations of T and B cells. However, they suggest the desirability of simultaneous testing by DNA hybridization and McAbs in future studies of lymphoma categorization.

DIAGNOSIS BY CLONALITY

T cells lack surface markers of clonality similar to SIg light chains (discussed in Chapter 7). T cell receptor genes normally undergo rearrangement during intrathymic development, permitting a wide diversity of idiotypic T cell receptors necessary for antigen recognition. A clonal population of T cells will produce a detectable band of rearranged DNA when labelled with DNA probes. In this manner, T cell clonality has been identified in skin affected by mycosis fungoides and in lymph nodes of MF patients labelled as "dermatopathic" (Weiss, et al., 1985). Although this is a promising and sensitive technique, the following areas need to be addressed:

1) The number of other cutaneous disorders studied needs to be amplified as controls have been extremely limited.

2) The predictive value of the demonstration of clonal T cell populations in skin is presently uncertain. Clonality has been demonstrated in 5 of 6 cases of lymphomatoid papulosis (Weiss, et al., 1986) and 3 of 3 cases of pityriasis lichenoides et varioliformis acuta (Weiss, et al., 1987). Examination of the photomicrographic

illustration of pityriasis lichenoides in the second study (Weiss, et al., 1987) suggests these cases could be included under the category of lymphomatoid papulosis type B (Willemze, et al., 1982b) or lymphomatoid pityriasis lichenoides (Black and Wilson Jones, 1972). Approximately 10% of lymphomatoid papulosis cases develop into lymphoma (Weinman and Ackerman, 1981). Development of lymphoma from classic pityriasis lichenoides is regarded as exceedingly uncommon even when one includes cases with cytologic atypia (McMillan and Everett, 1982b). The development of lymphoma in 3 of 3 randomly selected cases is very unlikely. If the cases of pityriasis lichenoides described by Weiss et al. are really lymphomatoid papulosis, this would indicate clonality in 8 of 9 cases. If one excludes them, clonality was present in 5 of 6 cases of lymphomatoid papulosis. Although no follow-up data are available, this small study group, therefore, suggests that clonality may be more common than overt malignancy (usually 10%) in these patients. If this is substantiated, then host immunity (Page 261, Chapter 8) may form part of the equation in determining final outcome. At any rate these data suggest that clonality like cytologic atypia is more common than clinical malignancy.

False negative results may also be a problem as certain peripheral T cell lymphomas (including 1 cutaneous lymphoma) may lack detectable T cell receptor gene rearrangement (Weiss, et al., 1988).

Clearly, follow-up data from these interesting studies are required.

C. THERAPEUTIC APPLICATIONS OF IMMUNOPHENOTYPING

1) CATEGORIZATION OF LYMPHOPROLIFERATIVE DISORDERS

The results here (Chapters 5, 6) and elsewhere have shown that when the diagnosis of lymphoma is already established, cutaneous lymphoproliferative disorders may be classified into various subtypes. This process rests on a more solid base when multiple markers are used (Chapter 6). The assortment of phenotypes formed is illustrated in Table 16. The OKT3+, Leu1+, OKT4+, Leu3A+ phenotype of CTCL (Chapter 5) contrasts with the OKT6+ Leu3A+ phenotype of Histiocytosis X (Chapter 7) and the variety of phenotypes in cutaneous lymphomas of non-MF/SS type (described in Chapter 6).

Improved categorization of lymphomas may eventually permit more rational therapy. B cell and T cell lymphomas of similar morphology appear to respond differently to chemotherapeutic regimens, with T cell lymphomas generally being less responsive to many of the schedules currently used for B cell lymphomas (Levine, et al., 1974). The development of anti-T cell drugs such as Tilorone hydrochloride (Levine, et al., 1974; Crotty, and Winkelmann, 1982) and deoxyadenosine/deoxycoformycin (Matsumoto, et al., 1982) suggests that immunologic typing will be useful in formulating therapeutic protocols. The results of

TABLE 16
PHENOTYPIC PATTERN PRESENT IN CUTANEOUS LYMPHOHISTIOCYTIC INFILTRATES

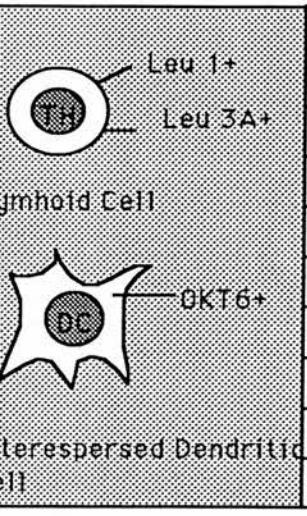
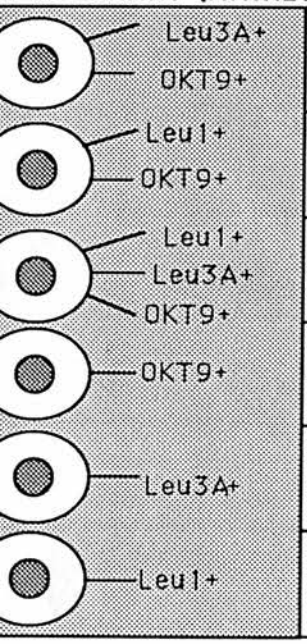
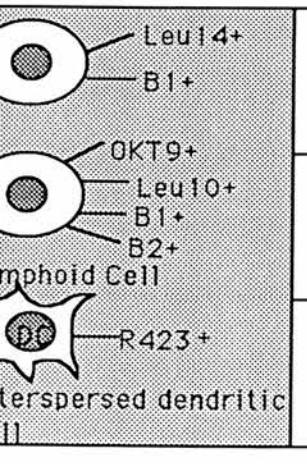
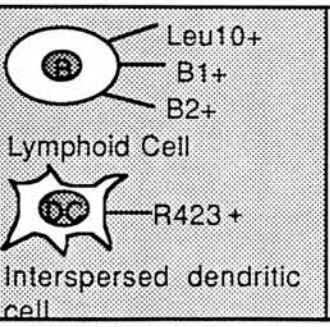
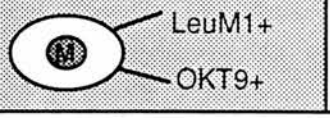
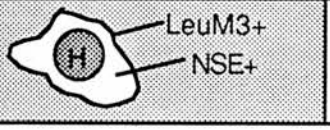
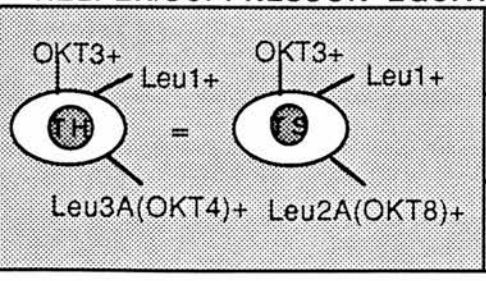
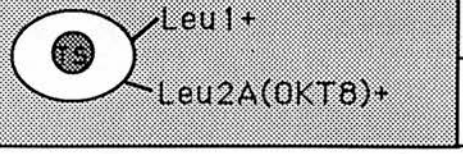
. MATURE T HELPER	DIAGNOSIS	No. of Cases Showing Phenotype
	Diffuse small cell well differentiated.	1
	Mycosis fungoides	20
	Sezary Syndrome	3
	Large plaque atrophic parapsoriasis	14
	Benign chronic dermatoses	4
	Lichen planus	3
	Lymphomatoid papulosis type B	1
	Allergic Contact Dermatitis	13
	Chronic lymphocytic leukaemia	1
	Lymphocytic infiltrate of Jessner	1
2. ABERRANT (MAINLY T)		
	Diffuse large cell lymphoma	2
	Diffuse large cell lymphoma	1
	Diffuse large cell lymphoma	2
	Diffuse large cell lymphoma	1
	Diffuse large cell lymphoma Mycosis fungoides	1 3
	Diffuse small cell well diff.	1
ABERRANT B		
	Nodular mixed lymphocytic histiocytic Diffuse large cell lymphoma	1 1
	Diffuse large cell lymphoma	1

TABLE 16 (CONTINUED)

. Mature B Cell		
	Lymphocytoma Cutis	3
. MONOCYTE		
	Myelomonocytic Leukaemia	1
HISTIOCYTE (ABERRANT LANGERHANS' CELL)		
	Histiocytosis X	3
MACROPHAGE (HISTIOCYTE)		
	Sarcoidosis	1
LARGE ATYPICAL CELL (AND REED STERNBERG LIKE CELL)		
	Lymphomatoid Papulosis type A	2
HELPER/SUPPRESSOR EQUIVALENCE		
	Benign chronic dermatosis	1
	Large plaque atrophic parapsoriasis	2
	Lichen Planus	1

10. MATURE T SUPPRESSOR PHENOTYPE

	Large plaque atrophic	
	Parapsoriasis	1

NOTE:

a) + denotes positive staining of ≥ 50% of lymphoid infiltrate (or of histiocytic/monocytic component in Histiocytosis X, sarcoidosis, lymphomatoid papulosis type A, chronic myelomonocytic leukaemia).

b) Phenotype 1 (Mature T Helper) has also been described in lymphomatoid granulomatosis (Harrist et al., 1981; Harrington, et al., 1983) and psoriasis (Baker, et al. 1984).

c) Phenotype 10 (Mature T Suppressor) has been described in pityriasis lichenoides et varioliformis acuta (Wood, et al., 1987), pityriasis lichenoides chronica (Wood, et al., 1987), erythema multiforme (Margolis, et al. 1983) and graft versus host disease (Gomes, et al., 1982).

phenotyping may also have prognostic significance (Yamanaka, et al., 1981).

2) REDEFINITION OF DISEASES

Mature T cell markers may also be used to redefine immunologically certain disorders within the CTCL complex. For instance, cases of pagetoid reticulosis (considered by many to be a localized variant of mycosis fungoides) can either demonstrate the OKT8+ (suppressor) or OKT4+/Leu3A+ (helper) phenotype (Catovsky, et al., 1982; Mackie and Turbitt, 1984).

3) PROGNOSIS

Antibodies to the transferrin receptor may also be useful prognostically as transferrin receptor expression may correlate with DNA synthesis rates, histologic grading and survival in non-Hodgkin's lymphoma (Habeshaw, et al., 1983; Kvaloy, et al., 1984).

The loss of Leu1 reactivity may be associated with a worse prognosis in CTCL.

The expression of lambda light chain in CBCL has been associated with a poorer response to treatment and the tendency for a particular tumour to fall into the high grade category (Lauder, et al., 1985).

4) INFERENCES FROM DENDRITIC MARKERS AND VIRAL ASSOCIATED ANTIGENS

The OKT6 marker may be useful in identifying cutaneous T cell lymphoma when loss of mature T cell markers has occurred. OKT6 reactive cells are present in the dermal

infiltrates of cutaneous T cell lymphoma and benign T cell infiltrates (Chapters 4, 5, 6, 7). B cell lymphomas in contrast are devoid of an OKT6 positive component (Chu, et al., 1982a; Haynes, et al., 1982b) (Chapter 6) but may contain an R423 positive dendritic component (Table 14). Further studies are required to determine if this is a consistent finding.

The presence of the OKT6 marker on the histiocytic cells of Histiocytosis X (Chapter 7) suggests OKT6 labelling may be a useful adjunct in the diagnosis of that disorder.

Although not covered experimentally in this thesis, it should be mentioned that T cell McABs have also been used to evaluate patients with retrovirus - associated adult T cell leukaemias and lymphomas. The clinicopathologic features of this group indicate that they are distinct from other lymphomas (Gallo and Wong-Stall, 1982). Patients with MF and SS are generally negative for antibodies against the pathogenetic human T cell leukaemia/lymphoma virus (HTLV). Monoclonal antibodies against HTLV have been produced and should be useful for studying the prevalence of HTLV in various immunoproliferative states. Preliminary monoclonal typing of adult T cell leukaemia cells has shown an OKT3+, OKT4+, Leu1+, Leu3A+ phenotype (Hattori, et al., 1981; Catovsky, et al., 1982; Nakahara, et al., 1982; Yamada, 1983).

5) MONOCLONAL ANTIBODY THERAPY

Lymphoma phenotyping should also have application in therapeutics. For instance, the Leu1 positivity of CTCL (Chapter 5), T101 positivity of CLL and J5 positivity of non-T ALL have been utilized by infusing the respective antibodies with resultant temporary clinical improvements (Ritz and Schlossman, 1982). However, phenotypic heterogenity including the presence of Leu1- subtypes of CTCL (Chapter 5) suggests difficulty in using this single agent with consistent success. Cross reactivity of Leu1 and T101 with normal tissue components has been a problem (Ritz and Schlossman, 1982) and could be predicted from results in Chapter 4.

The demonstration of OKT6, OKT9 and OKT10 reactivity in a component of the lymphoid population in CTCL (Chapter 5) raises the possibility of using these antibodies linked to chemotherapeutic agents, plant toxins (eg., Ricin), coated lipid vesicles, and radiopharmaceuticals to reduce various fractions of the neoplastic population. It should be emphasized, however, that the number of cells reacting with these antibodies varies in individual cases and is often small (Chapter 5).

Of course, researchers with an embryologic point of view might argue that if these cells are "dedifferentiated" or "immature", they are the most important ones to eliminate. Even if OKT9, 5E9 expression is due to proliferation rather than a "block" or "reversal" in

differentiation, it could still be argued that it is crucial to eliminate this proliferating compartment. Examination of the normal tissue distribution of these determinants will be required as a necessary preliminary to such trials. The extrathymic distribution of the OKT6, OKT9, and OKT10 determinants, for instance, has been demonstrated here (Chapter 4). Passive therapy with these antibodies might, therefore, only be appropriate in advanced cases of CTCL in which normal lymphoid architecture has been largely destroyed. The results with the myeloid antibodies (Chapter 4) are of interest in this respect as some of them appear to have a very limited tissue distribution. Other problems associated with McAb therapy have recently been outlined (Estabrook, and Patterson, 1983).

6) STIMULATION OF IMMUNE REACTIVE CELLS

The prospect of pharmacologically manipulating the HNK1+ lymphocytes, T cells, and macrophages present in lymphoma specimens (Chapter 5, 6) (McMillan and Stoneking, 1987) is intriguing. For instance, interferon, retinoic acid or interleukin 2 may stimulate K/NK activity (Pattengale, et al., 1982; Kessler, et al., 1983; Lanier, et al., 1985).

The prevalence of HNK1+ lymphocytes in 73 biopsies of benign and malignant cutaneous lymphohistiocytic infiltrates (and control scalp skin) is shown in Table 17.

The functional capability of such cells is, however,

TABLE 17-PREVALENCE OF HNK1+ LYMPHOCYTES IN BENIGN AND MALIGNANT CUTANEOUS LYMPHOHISTIOCYTIC INFILTRATES

DISEASE	NO. POSITIVE/NO. STUDIED (TOTAL =71)	% OF POSITIVE CELLS
CTCL	14/27	≤1%-5%
1. Mycosis fungoides	8/18	≤1%-5%
2. Sezary's syndrome	3/3	≤1%
3. Non-MF/SS type	3/6	1-5%
CBCL (1 nodular, 2 diffuse large cell)	2/3	5-10%
CNCL (difuse large cell)	1/2	<1%
Cutaneous Hodgkin's disease	1/1	15%
Lymphomatoid papulosis	3/4	<1-10%
Large plaque parapsoriasis	2/9	≤1%
Lichen planus	2/3	≤1%
Allergic contact dermatitis	1/7	<1%
Sarcoidosis	0/1	0%
Histiocytosis X	2/3	≤1%
Myelomonocytic leukaemia	0/1	0%
Lymphocytic infiltrate of Jessner	0/2	0%
Atopic dermatitis	0/3	0%
Normal skin (scalp)	0/5	0%

unknown. Subpopulations of HNK1+ lymphocytes with varying cytolytic potential may be identified through their expression of HNK1 (Leu7) and Leu11 (NK-15) antigens (Lanier, et al., 1983). The HNK1+ population may also be subdivided into subsets with positive or negative expression of T subset markers (OKT4, OKT8), the various fractions identified having different tissue distributions (Pizzolo, et al., 1984). Indirect evidence concerning the cytotoxic capacity of various tumour immune cells may, therefore, be obtained by extensive analysis of their antigenic expression. Similar methods may be applied to macrophages, as subsets of the mononuclear phagocyte system become better defined.

Finally, a more pragmatic approach would be to quantify such cells in tumour specimens and correlate their presence with clinical response to immunomodulatory therapy.

The conceptual and practical difficulties in identifying K/NK cells in human tumours and utility of the data generated has recently been debated (Manara, et al., 1988; McMillan, 1988).

II. SUMMARY

With reference to the objectives outlined in Chapter 2, pages 20-23, the results may be summarized as follows:

OBJECTIVE

1 a) Monoclonal determinants expressed on T cells (and T subsets), B cells of varying maturity, K/NK cells,

monocytes/macrophages, and D cells are readily demonstrable in non-malignant lymphoid tissue (tonsil, lymph node, thymus) and skin (Chapter 4).

The expression of the determinants tested is limited in skin but they are readily demonstrated in benign and malignant cutaneous lymphoid infiltrates (Chapters 4-7)

b) The determinants examined have characteristic topographic distributions (chapter 4).

c) So-called "immature determinants" (OKT6, OKT9, OKT10) are not restricted to thymus and bone marrow, contrary to the indications from prior studies of these markers.

The OKT6 determinant is present on dendritic cells in skin, tonsillar crypt epithelium, and in cells occupying the interfollicular areas of some non-malignant lymph nodes (chapter 4). The nature of these OKT6+ cells is discussed in Chapter 4. The OKT6 marker is also commonly present on the dendritic component of benign and malignant cutaneous lymphoid infiltrates of T cell type (chapter 4-7). (B cell infiltrates in contrast contain an R423+ dendritic cell, Chapters 6, 7).

The OKT9 and OKT10 determinants are present in non-malignant peripheral lymphoid tissue (tonsil, Chapter 4; lymph node not tested), and in benign (OKT9) and malignant (OKT9, OKT10) cutaneous lymphocytic infiltrates (Chapters 5, 6, 7).

2) Allergic contact dermatitis, a prototype of reactive

inflammatory states, exhibits a mature helper T cell phenotype (Leu1+, OKT3+, Leu3A+, OKT4+, OKT9-, OKT10-) with interspersed OKT6+ dendritic (Langerhans') cells (Chapter 4). This phenotype is also typical of a variety of inflammatory disorders (see Discussion section, this chapter).

3) Concerning Mycosis Fungoides/Sezary Syndrome (CTCL)

a) a mature helper T cell phenotype (Leu1+, OKT3+, Leu3A+ OKT4+ (Chapter 5) in CTCL contrasts with acute T cell lymphoblastic leukaemia and lymphoblastic lymphoma which usually show immature (early or common thymocyte) phenotypes.

An admixture of suppressor cells (Leu2A+, OKT8+) is also present. The hypothesis that CTCL might be readily diagnosed through the demonstration of a phenotypically monomorphous helper infiltrate is, therefore, not substantiated.

b) cells expressing the immature antigens OKT9, OKT10 are present in the cutaneous infiltrates,

and

c) comparison with the prelymphomatous disorder, large plaque parapsoriasis/poikiloderma vasculare, et atrophicans (mature T helper) and reactive states, eg., allergic contact dermatitis, atopic dermatitis (also mature T helper) indicates preferential expression of the immature determinants OKT9 and OKT10 in CTCL and other cutaneous

lymphomas (Chapters 5 and 6).

OKT9 expression in the parapsoriasis group is heterogeneous in addition to being less frequently found than in CTCL (Chapter 5).

The overlap in helper/suppressor ratios in benign prelymphomatous, and lymphomatous disorders (Chapters 4, 5) further supports the view that testing of cutaneous lymphocytic infiltrates with the mature subset markers used here is of limited diagnostic value.

4) Cutaneous lymphomas of non-MF/SS type

a) may be categorized with McAbs into T or B types (Chapter 6).

No true histiocytic lymphoma is identified here. Unclassifiable (U cell) types occur as with previous methods. The inclusion of multiple markers decreases the number of unclassifiable cases (Chapter 6).

b) show evidence of aberrant differentiation with the presence of phenotypes not found during normal T cell or B cell ontogeny.

5) Characteristic antigenic patterns are found in other cutaneous disorders involving mononuclear-phagocytic cells, eg., sarcoidosis, histiocytosis-X, myelo-monocytic leukaemia (Chapter 7). The histiocyte of histiocytosis X, in common with the normal Langerhans' cell, expresses the OKT6 determinant, but in addition reacts with the Leu3A (helper T cell) antibody.

The immuno-architectural pattern of the dermal infiltrate of lymphocytoma cutis taken in conjunction with overlying epidermis (Chapter 7) is remarkably similar to tonsillar lymphoid tissue with its adjacent crypt epithelium (Chapter 4) or B and T cell zones of human lymph node.

Lymphomatoid papulosis demonstrates phenotypic diversity which may be useful prognostically.

6) Putative immune cells, i.e., HNK1+ lymphocytes (probably K/NK cells), macrophages, and non-neoplastic T cells are present in cutaneous lymphomas (Chapters 5, 6), prelymphomas (Chapter 5), pseudolymphomas (Chapter 7) and inflammatory states (Chapters 4, 5, 6). The prevalence of HNK1+ lymphocytes in benign and malignant cutaneous lymphohistocytic infiltrates is summarized in Table 17. Their presence in lymphoma may be of prognostic or therapeutic import.

7) The Be1/Be2 antigens purported to be specific for lymphomas are present in

a) non-malignant lymphoid tissue (Be2 in tonsil, Chapter 4),

b) skin (Be1 in follicular epithelium, Be2 in endothelial cells, Chapter 4),

c) benign lymphocytic infiltrates (Chapter 5),

d) pseudolymphomas (Chapter 7), as well as cutaneous lymphomas of MF/SS (Chapter 5)and non MF/SS (Chapter 6) types.

They are preferentially expressed in lymphomas (in terms of numbers of cases showing Be1 and/or Be2 positivity). Overlap with benign disorders (in terms of percentages of positively staining cells and frequency of positively reacting cases) makes their present application in tissue section diagnosis impractical. The reactivity of Be1 and Be2 with a variety of non-malignant and lymphomatous cell populations is summarized in Table 15.

Within the limits of sensitivity of the indirect immunoperoxidase method used, non-malignant lymphoid tissue (tonsil) is non-reactive with the J5 (CALLA) marker. Rare cases of cutaneous lymphoma are J5 positive (Chapters 5). No comparison with benign lymphocytic infiltrates is presently available.

III. CONCLUSION

One decade ago, it was possible to extract lymphocytes from tissues and make a gross assessment of T or B content by rosetting assays. The development of McAbs has permitted a more accurate dissection of a wide range of normal and malignant lymphoid populations (Chapters 4-7). The studies described here with McAbs on tissue sections indicate that lymphomas are often more complex than the simple expansion of a clone of cells. The inter-relationships of the constituent cell types is wide open to speculation and further investigations. These reagents provide the framework for more creative diagnostic, prognostic and therapeutic

approaches to cutaneous lymphoma. They offer the exciting possibility of being able to treat lymphoma patients more effectively. The studies here, however, demonstrate conclusively that any new marker must be extensively tested in non-malignant lymphoid tissue, reactive and malignant states prior to its clinical application.

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TABLE 2

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	SPECIFICITY
T Cell and Subsets	
OKT3 (CD3)	Pan T (Kung, et al, 1979).
OKT4 (CD4)	Helper T (Ledbetter, et al,1981).
OKT8 (CD8)	Suppressor T (Kung, et al, 1979).
Leu1 (CD5)	Pan T (Ledbetter, et al,1981).
Leu2A (CD8)	Suppressor T (Ledbetter, et al,1981).
Leu3A (CD4)	Helper T (Ledbetter, et al,1981).
Leu4 (CD3)	Pan T (Ledbetter, et al,1981).
Leu5 (CD2)	E receptor (Ledbetter, et al,1981).
T11 (CD2)	E receptor (Ip, et al, 1982; Greaves, et al, 1981).
OKT11A (CD2)	E receptor (Greaves, et al, 1981).
T101 (CD5)	Pan T, some B cell neoplasia (Royston, et al, 1980).
3A1 (CD7)	Suppressor T cells, subset of helper T cells (Cossman, et al, 1983), K cells (Herberman and Ortaldo, 1981).

TABLE 2 (Continued)

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	SPECIFICITY
Immaturity Antigens	
OKT6 (CD1a)	Immature (common) thymocytes (Kung, et al, 1980), epidermal dendritic cells (Chapter 4).
OKT9	Immature thymocytes, T cell ALL (Kung, et al, 1980), wide range of proliferating cell types in culture (Greaves,et al, 1981), eg., mammary carcinoma, teratocarcinoma. Transferrin receptor.
OKT10 (CD38)	Immature thymocytes (Kung, et al, 1980), bone marrow progenitor cells some activated T cells (Kung, et al, 1980), K cells (Herberman and Ortaldo, 1980).
Killer/Natural Killer	
HNK1(Leu7)	Granular lymphocytes with K/NK function (Abo and Balch, 1981),fetal hamster olfactory epithelium, (Ward, 1986) human neuroectodermal tumours, (Cailland,et al, 1984) neural and neuroectodermal cells of several species (Lipinski, et al,1983; Schuller-Petrovic, et al, 1983).
B Cell	
B1 (CD20)	B cells, B cell lymphomas (Nadler, et al, 1981a).
B2 (CD21)	Subset of B cells and B cell lymphomas (Nadler, et al, 1981b).
Leu14 (CD22)	B cells, weakly with monocytes, but not macrophages.
Kappa	Anti-kappa light chains.
Lambda	Anti-lambda light chains

TABLE 2 (CONTINUED)

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	**SPECIFICITY**
HLAD Locus	
HLADR	Monocytes, macrophages, Langerhans' cells, activated T cells, B cells (Warnke and Levy, 1980).
Monocyte/Macrophage/D Cell	
OKM1 (CD11)	Monocytes, macrophages, myeloid series (Breard, et al, 1980).
MO2 (CD14W)	Monocytes, macrophages (Todd, et al,1981).
LeuM1 (CD15)	Monocytes, macrophages, myeloid series (Hanjan, et al, 1982) adenocarcinomas, (Sheibani, et al, 1986), interdigitating reticulum cells, (Hofman, et al, 1984), Reed-Sternberg cells, (Pinkus, et al, 1985; Hsu, et al 1986).
LeuM3 (CD14)	Monocytes, macrophages (Dimitriu-Bona, et al, 1983).
My3	Monocytes (Civin, 1983).
Acid Alpha Naphthyl Acetate (Non-specific) Esterase	Macrophages ("Histiocytes") (Yam and Li, 1971)
R423	Dendritic reticulum (follicular dendritic) cells (Naiem, et al, 1983).
OKT6 (CD1a)	Epithelial dendritic cells (Chapter 4)
Non Lineage Restricted	
2D1 (CD45)	T200 Leukocyte common antigen (Pizzolo et al 1980).

Table 2 (Continued)

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	SPECIFICITY
Common Acute Leukaemia	
J5 (CD10)	Common acute lymphoblastic leukaemia antigen, (Ritz, et al, 1980), adult and fetal nonhaematopoietic tissue (Metzgar, et al, 1981) , immature T cells (Hsu, et al, 1985), neutrophils (Cossman, et al, 1983), germinal centre B cells, (Hsu, et al, 1984), B cell lymphomas and leukaemias (Hsu, et al, 1984; Cossman, et al, 1984).
Myeloblastic Leukaemia cell lines	
My10 (CD34)	Kg-1, Kg-1 a cell lines, some ALL and ANL, myeloid progenitor cells (Civin, et al, 1983, 1984).
My11 (CD45R)	Kg-1, Kg-1 a cell lines, monoblastic, B-lympho-blastoid lines, E rosette+ and E rosette- PBL, monocytes, some ALL, ANL, CLL, CFU-C but not CFU-E or BFU-E (Civin, et al, 1983, 1984).
My12	Kg-1, Kg-1a, U-937, K-562, HEL cell lines, some ANL, CML, ALL (Civin, et al, 1983, 1984).
My13	Kg-1, Kg-1 a cell lines blast cells from less than 10% of ANL or ALL, CLL. (Civin, 1983)
Leukaemia/Lymphoma	
Be1	Leukaemic CTCL lymphocytes, lymph nodes infiltrated by CTCL, Epstein Barr virus transformed cell lines and some long term T cell lines.
Be2	Peripheral blood lymphocytes from 75% of CTCL patients, Epstein Barr virus cell lines, some T cell lines and a subpopulation of lymphocytes from 5 of 8 patients with B cell chronic lymphocytic leukaemia

Table 2 (Continued)

SPECIFICITIES AND SOURCES OF MONOCLONAL ANTIBODIES AND ENZYME CYTOCHEMISTRY	
ANTIBODIES	**SPECIFICITY**
Control	
MOPC21	No known specificity.
Mouse Ascites	No known specificity.

NOTES OF EXPLANATION:

1. KG-1 is a myeloblastic leukaemia cell line derived from a patient with nonlymphocytic leukaemia (Koeffler, and Golde, 1978). The KG-1a cell line arose from it as a spontaneous tissue culture variant (Koeffler, et al., 1980). KG-1a cells are phenotypically less differentiated than KG-1 cells, and have the morphological and cytochemical features of primitive haematopoietic blast cells. (Koeffler, et al., 1980).

2. The McAbs MY10-13 have been raised against determinants on KG-1a cells. None of these antibodies react with blood granulocytes, red blood cells, or platelets from normal donors. Large subsets of peripheral blood lymphocytes and monocytes express the My11 antigen. My10, 12, 13 antigens are not expressed on significant numbers of blood lymphocytes or monocytes. My11 is found in 25% of bone marrowcells of normal donors. My10, 12, 13 bind only subpopulations of normal marrow cells.

3. Sources of McAbs:

A. Orthoimmunobiology. Raritan, New Jersey, USA: OKT3, 4, 6, 8, 9, 10, 11a, and OKM1.

B. Becton Dickinson Facs Systems Ltd., Sunnyvale, California, USA: Leu1, 2A, 3A, HLADR, LeuM1, LeuM3, Leu14, Anti-Kappa, and Anti-Lambda.

C. Coulter Ltd., Bethesda, Maryland, USA: B1, B2, T11, M02, and J5.

D. Hybritech, Inc., T101.

E. Barton Haynes, M.D., Dept. of Medicine, Duke University, North Carolina, USA: 3A1.

F. Toru Abo, Ph.D./Charles Balch, M.D. Departments of Microbiology and Surgery, University of Alabama, Birmingham, Alabama, USA: HNK1

G. David Mason, M.D., Department of Haematology, John Radcliffe Infirmary, Oxford, England: R423.

H. Curt Civin, M.D., Department of Paediatric Oncology, The John Hopkins Oncology Center, Baltimore, Maryland, USA: MY3, MY10-13, M.O.P.C. 21.

I. Carole Berger, Ph.D., Department of Dermatology, Columbia College of Physicians, New York, New York, Be1 and Be2.

J. American type Tissue Collection, Rockville, Maryland: M.O.P.C. 21. Mouse myeloma protein (culture supernatant with no known specificity, IgG1 Kappa produced by the P3 x63 AG B cell line.

4. ABBREVIATIONS

ALL: acute lymphoblastic leukaemia; CLL: chronic lymphocytic leukaemia; PBL: peripheral blood lymphocytes; BFU-E: burst forming units - erythroid; CFU-E: colony-forming units erythroid; CFU-C colony forming units - culture; ANL, acute nonlymphocytic leukaemia.